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OF THE

CAROLINE BREWER CROFT CANCER COMMISSION

OF

THE HARVARD MEDICAL SCHOOL

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FEBRUARY 15, 1905.

DEAR SIR:

The Third Report of the Cancer Commission of the Caroline Brewer Croft Fund is herewith submitted for your consideration. Should you desire to receive the future reports of this Commission as they may be issued, will you kindly acknowledge the receipt of this one, and send your full address to the Secretary at your earliest convenience?

Very truly yours,

J. COLLINS WARREN, *Chairman,*
For the Caroline Brewer Croft Fund
Cancer Commission.

THE MEDICAL SCHOOL
OF
HARVARD UNIVERSITY
BOSTON, MASSACHUSETTS

THE THIRD REPORT

OF THE

CAROLINE BREWER CROFT CANCER COMMISSION

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THE HARVARD MEDICAL SCHOOL.

A CONTRIBUTION TO THE CLASSIFICATION OF TUMORS.[1]

F. B. MALLORY, M.D.

(From the Sears Pathological Laboratory of the Harvard University Medical School and the Pathological Laboratory of the Boston City Hospital.)

This paper represents the beginning of an attempt at a classification of tumors based on a more careful study of the histological differentiation of their cells and intercellular substances than has as yet been made. What is reported here is the result of a study of three kinds of fibrils produced by normal and tumor cells, and of the bearing of these fibrils on the classification of the tumors containing them. These particular tumors fall into three groups, and are selected for report at the present time partly because of their morphological relationship to each other, partly because they have yielded the most material for study, and because the histological staining methods used have proved more productive with them than with other tumors. The work is by no means complete, but the results obtained are of value and point the way for further investigation.

The three kinds of fibrils studied are the neuroglia fibrils of neuroglia cells, the myoglia fibrils of smooth muscle cells, and the fibroglia fibrils of connective tissue cells. Of these three kinds of fibrils the neuroglia fibrils have been the longest known and most studied. The myoglia fibrils have only recently attracted much attention, and the fibroglia fibrils were announced as discovered but little over a year ago.

These three kinds of fibrils have this feature in common,—that in the middle of their course they are for a greater or

[1] Received for publication Nov. 1, 1904.

less distance in intimate contact with the surface of the protoplasm of the cell which produced them. Away from their own cell they may or may not be in contact with the surface of other cells. Other points wherein these fibrils resemble and differ from each other will appear farther on in the paper.

Early in the work it was found that little use could be made of tissues which were not obtained directly from surgical operations and immediately (*i.e.*, within a few minutes after the operation) cut into very thin sections (two to four millimeters) and hardened in appropriate fixing solutions. The reason of this is that these fibrillar substances undergo degenerative changes very quickly. For example, the myoglia fibrils lose their power to stain differentially much more quickly even than neuroglia fibrils which are notoriously difficult to preserve perfectly. On this account, when the various differential stains for neuroglia fibrils were tested on the two other kinds of fibrils, it was found necessary to use tissue which was perfectly fresh when fixed, or very erroneous conclusions were drawn.

The following staining methods were found the simplest and most suitable to use in this investigation:

METHOD I.

Anilin Blue Stain for the Intercellular Connective Tissue Fibrils.

1. Zenker fixation; paraffin or celloidin embedding.

2. Transfer sections from water to a one-tenth per cent aqueous solution of acid fuchsin for five minutes.

3. Transfer sections directly (without washing) to the following stain for two to ten minutes:

Anilin blue soluble in water (Grübler)	0.5 gram.
Orange G (Grübler)	2.0 "
One per cent aqueous solution of phosphomolybdic acid . .	100.0 cc.

4. Differentiate and dehydrate quickly (rarely over one to two minutes) in two or three changes of ninety-five

per cent alcohol followed by absolute alcohol for paraffin sections.

5. Clear in xylol (filter paper blotting method for celloidin sections) and mount in xylol balsam.

Fibrin and neuroglia, myoglia, and fibroglia fibrils red, intercellular connective tissue fibrils blue, elastic fibrils yellow or pale pink. For embryonic tissue and for very rapidly growing sarcomata it is sometimes advisable to increase the strength of the acid fuchsin solution.

METHOD 2.

Stain for Fibroglia Fibrils.

1. Zenker fixation; paraffin or celloidin embedding.

2. Transfer sections from water to a one-tenth per cent aqueous solution of acid fuchsin for five minutes.

3. Transfer sections directly (without washing) to the following solution for two to ten minutes:

Orange G (Grübler)	2.0 grams.
One per cent aqueous solution of phosphomolybdic acid .	100.0 cc.

4. Differentiate and dehydrate quickly (rarely over one to two minutes) in two or three changes of ninety-five per cent alcohol followed by absolute alcohol for paraffin sections.

5. Clear in xylol (filter paper blotting method for celloidin sections) and mount in xylol balsam.

This method is identical with the preceding one with the exception that the anilin blue is omitted. The intercellular connective tissue fibrils are at first almost colorless, but after exposure to diffuse daylight for several days they turn pale to deep bluish green in color, owing to the effect of light on the phosphomolybdic acid. The red-staining fibrils are brought out with greater sharpness than in Method 1. It is possible to combine this method with either Weigert's elastic tissue stain or with an alum hematoxylin nuclear stain or with both, provided they are used first.

METHOD 3.

Stain for Fibroglia Fibrils.

1. Zenker fixation; paraffin or celloidin embedding.

2. Transfer sections from water to a one per cent aqueous solution of acid fuchsin over night in the cold, or for twenty to thirty minutes in the paraffin oven (56° C.).

3. Wash quickly in water (not over five seconds). Water extracts acid fuchsin very quickly.

4. Differentiate in a one-fourth per cent aqueous solution of permanganate of potassium for twenty to forty seconds. This step must not be prolonged beyond the exact time needed or the section will be completely decolorized.

5. Wash quickly in water (not over five seconds).

6. Dehydrate in ninety-five per cent alcohol followed by absolute alcohol for paraffin sections.

7. Clear in xylol (filter paper blotting method for celloidin sections) and mount in xylol balsam.

Fibrin and fibroglia, myoglia, and neuroglia fibrils red, intercellular connective tissue fibrils brownish yellow to colorless, elastic fibrils bright yellow.

Of the four methods given here in detail, this is the most difficult to use so as to get perfect results, but with it and the following method the best results in some cases can be obtained.

METHOD 4.

Phosphotungstic Acid Hematoxylin Stain.[1]

1. Zenker fixation; paraffin or celloidin embedding.

2. Transfer sections from water to a one-fourth per cent aqueous solution of permanganate of potassium for ten to twenty minutes.

[1] *Phosphotungstic Acid Hematoxylin.*— When this formula was first published the phosphotungstic acid manufactured by Merck was not pure. It contained a trace of phosphomolybdic acid, and also some oxidizing agent which ripened hematoxylin at once The acid obtained since then sometimes oxidizes hematoxylin and sometimes does not. It is a simple matter, however, to ripen the solution at once.

Hematoxylin	0.1 gram.
Water	80. cc.
10 per cent aqueous solution of phosphotungstic acid (Merck)	20. cc.

Dissolve the hematoxylin in a little water by the aid of heat, and add it when cool to

3. Wash in water.

4. Place in a five per cent aqueous solution of oxalic acid for one to two hours.

5. Wash thoroughly in several changes of water.

6. Stain in phosphotungstic acid hematoxylin for twenty-four to forty-eight hours.

7. Wash quickly in water.

8. Dehydrate in ninety-five per cent alcohol followed by absolute alcohol for paraffin sections.

9. Clear in xylol (filter paper blotting method for celloidin sections) and mount in xylol balsam.

Fibrin and fibroglia, myoglia, and neuroglia fibrils blue; intercellular connective tissue fibrils pale reddish brown or colorless; the coarse elastic fibrils a pale blue. If, after step 6, the sections are placed in a strong alcoholic solution (ten to twenty per cent) of chloride of iron for one to several minutes, followed by thorough washing in water, the intercellular connective tissue fibrils are completely decolorized and the blue-stained fibrils take a somewhat deeper tint.

These four methods are by no means perfect, but they are comparatively simple, and when used with intelligence yield valuable results. It is to be noted that they all stain about equally well, fibroglia, myoglia, and neuroglia fibrils as well as fibrin.

In addition to these methods various other stains, including the differential stains for neuroglia fibrils and Benda's differential stain for myoglia fibrils, were employed. With the possible exception of Weigert's neuroglia method all the neuroglia methods yet devised stain fibroglia and myoglia fibrils more or less perfectly, provided perfectly fresh tissue

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the rest of the solution. If the solution does not stain, it may be ripened by the addition of either of the following oxidizing reagents: peroxide of hydrogen (U.S.P.), ·2 cubic centimeter or one-fourth per cent aqueous solution of permanganate of potassium, ten cubic centimeters.

The best results are obtained by using hematein ammonium instead of hematoxylin, and allowing the solution to ripen spontaneously for several months. Solutions a year or more old stain with great intensity.

If hematein ammonium is used instead of hematoxylin and it is desired to ripen the solution at once, use half the quantity of either of the oxidizing reagents mentioned above.

is used. It is impossible, therefore, at present to differentiate between these three kinds of fibrils by means of their staining reactions alone; their relation to the cell and their morphology must also be taken into consideration.

In the following pages I shall consider the three classes of tumors under the headings of the characteristic fibrils which they produce.

NEUROGLIA FIBRILS.

In regard to the subject of glioma in general I have little to say here except that gliomata should be treated as an entity and not confounded in any way with tumors of other origin. We should not, as is often done, restrict the term "glioma" to the slow growing tumors, and employ the term "gliosarcoma" for those which proliferate rapidly. To do so is confusing, because the glioma is of ectodermal origin, while the term "sarcoma" has long been used for certain rapidly growing tumors of mesenchymal origin. There may have been some justification in the past for the use of the term, when it was not always possible to determine the true nature of certain rapidly growing tumors of the central nervous system. It seems probable, however, that now, with improved technical methods, it will be possible in the future to determine definitely the origin of such tumors by a careful study of the differentiation of their cells and intercellular substances, provided that the tissue is obtained in a perfectly fresh condition and is hardened in proper fixing reagents. For it is useless to waste much time or thought on tissues not preserved under the very best of conditions; even then they offer sufficient difficulties.

I wish to report under this heading a remarkable glioma which originated subcutaneously over the coccyx, because its clinical course and its histological structure have a bearing on the classification of gliomata. The tumor gave rise to metastases in both groins, and its removal was followed by recurrences. A brief description[1] of the original growth has already appeared in print.

[1] Mallory. Journal of Medical Research, 1902, Vol. viii, pp. 1–10.

The tumor occurred in a woman who was forty-four years old at the time of the first operation in February, 1902. It had been present for twenty-five years to the patient's knowledge, and was probably congenital, because when first noticed it was a nodule the size of a "hickory-nut," situated in the median line of the back over the coccyx. It kept that form and size until about one year before removal, when it rapidly increased in size. The enlargement was particularly marked during the three months preceding the operation. At that time it had reached the size of a "baseball." It lay almost exactly in the median line over the coccyx and lower part of the sacrum. It was not attached to the underlying bony structure, and the skin covering it was freely movable.

The patient was operated on a second time June 17, 1903, for metastases in the right groin. A large mass measuring 6.5 centimeters in diameter and five centimeters in thickness and three smaller nodules were removed.

A third operation[1] was performed June 20, 1904, for recurrences over the coccyx and in the right groin, and for metastases in the left groin.

The mass over the coccyx was the size of an orange and showed the scar of the first operation on its surface. On section it consisted of one large nodule six centimeters in greatest diameter and of several smaller nodules. In each groin were numerous nodules, of which the largest measured four centimeters in diameter.

The general gross characteristics of the tumor masses at the times of the different operations and from the different locations were the same. They were all distinctly encapsulated, but the separate nodules from the same locality were usually closely bound together. In the groins, however, were a few smaller nodules, the size of beans, which were embedded in fat tissue. The nodules for the most part were fairly firm and elastic, but a few were somewhat soft. The consistence could be compared to that of a fibrosarcoma.

[1] Through the kindness of Dr. J. H. Sheahan, of Quincy, Mass., who had charge of the case from the beginning, I was able to be present at this operation and obtain the tissue in a perfectly fresh condition for histological preservation.

On section the surface was gray, translucent, and for the most part perfectly homogeneous. In some of the larger nodules, however, there were irregular areas of hemorrhage and of necrosis.

While the tumor in gross suggested the appearance of a sarcoma, the structure histologically is that of a carcinoma. It consists of large and small alveoli of cells of an epithelial type embedded in a fairly abundant connective tissue stroma. In the most rapidly growing parts of the tumor the cells in the alveoli are often round, but as a rule they are more or less oval or spindle-shaped. The outlines of the cells are not as a rule very sharply defined. The nuclei are round to oval in shape, vesicular in type, and stain rather lightly. They contain numerous fine and a few coarse chromatin granules, but no distinct nucleolus. Mitotic figures are numerous.

The remarkable feature about this case is that fibrils are present in varying number between the cells which fill the alveoli. In some places they are very abundant, but in others they are few in number. They vary in thickness, but for the most part are rather coarse. To some extent at least these coarse fibrils are due to the more or less intimate union of two or more fibrils. A few of the fibrils are straight, but most of them are wavy or crinkled; still others present a corkscrew-like appearance.

Sometimes the cells in the peripheries of the alveoli immediately adjoin the connective tissue stroma, but often they are separated more or less from it by a layer of their own fibrils. Sometimes these fibrils run parallel with the stroma, at other times they lie perpendicular to it and terminate in swollen ends which unite laterally to form a sort of limiting membrane, just as the neuroglia fibrils do in the cord of the embryo. The fibrils in the alveoli always run parallel with the long axis of the cells to which they belong. In the necrotic areas the fibrils still persist in places, although the necrotic cells are infiltrated with numerous polynuclear leucocytes.

In some of the tumor nodules, especially in those which have replaced the inguinal lymph nodes, the tumor shows

another type of growth, namely, it becomes distinctly papillary. The cells in such cases are arranged in one or more layers around delicate papillary stalks containing blood vessels and a little connective tissue. The nuclei are in the ends of the cells farthest away from the vessels. Between the cells are a few fibrils radiating out from the central stalk. The outer edge of the cells stains like the cuticular surface of epithelial cells in many glands, and shows two minute dots in the center of the surface.

In the smallest inguinal lymph nodes which were invaded the tumor cells extend along the peripheral sinuses in exactly the same way that metastases of carcinoma do, but everywhere they produce their own kind of fibrils. It is to be presumed that the cells which are carried from one lymph node to another to form metastases are young cells which are not held back by any fibrils which they have produced.

The true connective tissue stroma of the tumor varies considerably in amount, but for the most part is well developed and in places is abundant. In properly stained preparations the delicate, straight, or slightly curved fibroglia fibrils stand out in marked contrast with the coarse, wavy fibrils produced by the tumor cells within the alveoli.

It is difficult to determine exactly the relation of the fibrils within the alveoli to the cells which produce them, largely for the reason that until they reach a certain thickness and length the fibrils do not stain distinctly. It is, therefore, impossible to say whether they are formed first within the protoplasm of the cell and later are moved to the periphery, or, as seems more probable, are produced at the periphery in the beginning and gradually elongate in both directions beyond the limits of the cell. Certainly at a distance from the cell which produces them they run between other cells, and to some degree, therefore, may be said to be intercellular.

The proper diagnosis in this case seems unquestionably to be that of glioma. The only other cells known at present which produce fibrils which from their relation to the cell and from their staining reactions can be considered are the smooth muscle and connective tissue cells. But in the tumors

produced by such cells the arrangement is never alveolar, but the individual cells are separated from each other by intercellular connective tissue fibrils. In the case of the series of connective tissue tumors (fibroma, fibrosarcoma, spindle cell sarcoma) the fibrils are produced by the tumor cells. In the series of smooth muscle tumors (leiomyoma, malignant leiomyoma) the intercellular connective tissue fibrils, which surround to some extent every muscle cell, are produced by the connective tissue cells of the stroma and extend in between the muscle cells probably on account of a physiological demand for them. In a glioma the neuroglia cells and their fibrils grow in masses without any intercellular invasion by connective tissue cells and fibrils; the neuroglia and connective tissue are always distinct, and the same relation is true in regard to the neuroglia tissue in teratomata. Another point of value in the differential diagnosis is that the coarse wavy fibrils of this tumor are entirely different in character from the straight or slightly curved finer fibrils produced by smooth muscle and connective tissue cells.

There is one other possibility that should, perhaps, be mentioned. The epithelial cells of the skin produce in small numbers wavy, corkscrew-like fibrils which are called " epithelial fibrils." The possibility of a tumor of epithelial origin containing fibrils between the cells should, therefore, be borne in mind. In those carcinomata in which I have hitherto found epithelial fibrils, they were present in comparatively small numbers.

The type of neuroglia cell occurring in the tumor under consideration corresponds to that of early embryonic life. The cells are large and round, oval, and spindle in shape. The protoplasm is abundant, and the fibrils run parallel with the long axis of the cell. A tumor composed of cells evidently of much the same type as found in this case is reported by Muthmann and Sauerbeck.[1] It occurred in the fourth ventricle, but unfortunately they did not stain for neuroglia fibrils, owing to the fact that the tumor was preserved with

[1] Ziegler's Beiträge, 1903, xxxiv, 445–488.

reference to its gross relations instead of with a view to its histological differentiation.

So far as I am aware this is the first glioma reported as arising outside of the central nervous system and its outgrowths. Its histogenesis, however, as stated in the original report of the case, probably "admits of a simple explanation. In a paper on Sacro-Coccygeal Dimples, Sinuses, and Cysts,[1] published in 1892, I stated that examination of the tissues over the coccyx and sacrum of seven embryos showed in each of six of them one or more gland-like structures lined with epithelium. These I believed to be the remains of the lower end of the neural canal, which closes very irregularly. It seems to me that the explanation of the tumor described in this paper is connected with these same embryonic remains. These cells unquestionably have, in consequence of their origin, the potential possibilities of differentiating either into cells like the epidermis (as seen in the dermoid sinuses and cysts found in this region) or into ependymal cells and their derivatives, in this case neuroglia tissue."

This tumor has an interesting bearing on the classification of gliomata with regard to other tumors. As a rule the glioma is grouped with the new growths of mesenchymal origin, perhaps because the slow growing forms produce fibrils, while the rapidly growing ones resemble more or less closely the sarcomata. Certainly the neuroglia, smooth muscle, and connective tissue cells, with their peculiar differentially staining fibrils in intimate contact with the protoplasm, stand in close morphological relationship.

On the other hand this case shows how closely the glioma is related to the carcinoma. The alveolar arrangement of the tumor cells and the method of forming metastases are identical. The papillary form of growth is another point in common. The relationship is shown in another way in other gliomata in which many of the neuroglia cells differentiate into ependymal cells which line small cavities, just as the cells in the alveoli of a carcinoma of the breast often differentiate

[1] American Journal of the Medical Sciences, 1892, Vol. 103.

a cuticular surface and form small gland cavities. Gliomata, as a rule, do not suggest an alveolar arrangement. This is probably due to the fact that the connective tissue stroma is so slightly developed that it attracts little attention. A similar condition is sometimes present in carcinomata when the epithelial cells are greatly in excess. An alveolar arrangement is usually best marked when the stroma is fairly abundant.

This tumor emphasizes the fact that neuroglia cells behave like epithelium in their relation to connective tissue in spite of the fact that they produce fibrils of their own, *i.e.*, connective tissue does not surround each single neuroglia cell, but only masses of them. This relationship holds good both under normal conditions and in tumors, including teratomata. It would, therefore, be possible to group together, on the basis of their relationship to the stroma of blood vessels and connective tissue which nourish and support them, epithelial tumors (adenoma, adenocystoma, malignant adenoma, carcinoma), gliomata, and melanomata. The melanomata usually have a most typical alveolar arrangement, but often, especially when originating in the eye, suggest as little evidence of an alveolar arrangement as do most gliomata within the central nervous system.

MYOGLIA FIBRILS.

Under the term "leiomyoma" I shall include all the tumors which arise from smooth muscle cells, whether their rate of growth is extremely slow or very rapid. It is important that this group of tumors be treated as an entity just as much as those included under the term "carcinoma," in order that attention may be called to those characteristics which all the tumors of smooth muscle origin have in common.

For a clear understanding of this group of tumors it is necessary to state briefly those histological characteristics of a smooth muscle cell which enable us to distinguish it from other cells, because the diagnosis of a leiomyoma can be based only on the histological differentiation of the cells of which it is composed. In the past it has been based chiefly

on the following features: rod-shaped nuclei, spindle-shaped cells, sharply limited cell outline, deep staining of the protoplasm with acid dyes, and the arrangement of the cells. These points, however, are not sufficient for the identification of smooth muscle cells under all conditions.

The form of the smooth muscle cell varies much in different parts of the body, but in general it may be said to be spindle-shaped. At each tapering end it overlaps and is closely attached to other smooth muscle cells running in the same direction. The cells thus form slender columns composed of single rows of cells joined end to end. The columns of cells are usually grouped in bundles or layers. Each muscle cell contains a nucleus which is generally described as rod-shaped. In addition it possesses two kinds of fibrils, the "Grenzfibrillen" (Heidenhain)[1] or "myoglia fibrils" (Benda),[2] and the "Binnenfibrillen" (Heidenhain) or internal fibrils.

The myoglia fibrils are histologically the more important. They are situated at the periphery of the cell, lying in or on its limiting surface, and run parallel with its long axis. They do not end with the limits of the cell, but continue indefinitely along the cell column. A cross section of a smooth muscle cell shows about a dozen myoglia fibrils to a cell lying in the limiting membrane of its protoplasm. They are much more developed in some situations (vas deferens) than in others (arteries), but in general may be said to be distinctly coarser than the fibroglia fibrils of connective tissue cells. They can be demonstrated by a variety of staining methods, but no staining method has yet been found which will differentiate them from fibroglia fibrils. They degenerate so quickly that post-mortem material is practically useless for their study. Tissues from surgical operations immediately fixed in appropriate solutions are required. In regard to the function of these myoglia fibrils, Benda believes that they are not concerned in the contraction of smooth muscle cells, but have only elastic properties.

[1] Ergebnisse der Anatomie und Entwickelungsgeschichte, 1898, Vol. viii, pp. 3–11.

[2] Ergänzungsheft zum, xxi Band, 1902, des Anatomischen Anzeigers, p. 214.

The Binnenfibrillen, or internal fibrils, lie between the myoglia fibrils and the small amount of undifferentiated protoplasm which immediately surrounds the nucleus. It is doubtful if these fibrils, which are very fine, can be clearly demonstrated in mammalian muscle, although they have been seen in some of the lower forms of animal life. It is probable, however, that the deeper staining of the protoplasm of smooth muscle cells, with eosin and other acid dyes, is due, in part at least, to the presence of these fibrils, and the curious swollen appearance of the middle of smooth muscle cells in post-mortem tissues may possibly be due to post-mortem contraction of these fibrils.

We are indebted chiefly to Heidenhain for our knowledge of the structure of the normal smooth muscle cell. Benda has added certain differential staining methods (which, however, stain fibroglia fibrils equally well), and has given an interpretation of the functions of the two kinds of fibrils somewhat different from that offered by Heidenhain. No one, apparently, has applied the knowledge of the existence of these fibrils to the study of leiomyomata. The staining methods given in detail at the beginning of this paper were, therefore, applied to the study of as many of these tumors as it was possible to obtain immediately after removal by operation.

The study of a series of leiomyomata obtained chiefly from the uterus shows that while in general the smooth muscle cells closely resemble those found in normal tissues, they may vary considerably in form from the normal type. For example, a leiomyoma occasionally occurs in which the cells are very long and thin, and the nuclei are the slenderest of rods. In still other cases the cells are short and thick, and the nuclei have a long or short oval form. This difference in the shape of the cells depends, in part at least, on the rapidity of growth of the tumor. In general it may be said that the slowest growing smooth muscle cells are the most slender.

The myoglia fibrils in the tumors vary somewhat in number and coarseness, but always form a well marked and characteristic feature of the cells. As in normal tissues they

remain closely applied to the cell columns; so far as can be determined they do not leave the surface of cell protoplasm and mix with the intercellular connective tissue fibrils surrounding the smooth muscle cells. The myoglia fibrils show a certain tendency to twine together, especially in tissue which is somewhat edematous, so as to form what seem to be unusually coarse fibrils. This appearance is most marked at the ends of cells, where they are drawn out thin, so that the fibrils running from one cell to the next are brought into close apposition.

It was found easily possible to obtain a series of leiomyomata in which the rate of growth varied from extreme slowness up to the greatest rapidity, and thus compare with each other the smooth muscle cells proliferating at different rates of speed. The slowest growing forms have already been mentioned. In the somewhat more rapidly growing tumors (corresponding to the fibrosarcoma of connective tissue origin) in which only one or two mitotic figures can be found in a section, the cells are usually larger. They may preserve their usual form, or may be shorter and thicker, and the nuclei may vary from long to short oval in form. Occasionally large cells, with multiple or with large lobulated nuclei, occur. The myoglia fibrils are always well developed and sharply define the limits of the cell protoplasm.

In the most rapidly growing tumors of this series a spindle or oval form is usually retained by the cells, but in some tumors more or less rounded cells occurred, sometimes in numbers, and large cells with multiple or with large lobulated nuclei are common.

One tumor in the series deserves brief and especial mention. It was growing so rapidly that nearly every field under the oil immersion shows one to three or four mitotic figures. The tumor occurred in the uterus of a woman fifty-two years old, and was stated to be of about fourteen weeks duration. The uterus contained in its posterior wall a tumor the size of a "cocoanut." The periphery of the tumor and areas scattered here and there in it resembled the tissue of an ordinary leiomyoma, but the rest of the tissue was grayish, homogeneous,

and rather soft, resembling a sarcoma. Projecting into the uterus, but attached at its base to this tumor, was a second mass the size of an "orange" of a similar soft consistence. Histological examination of the two large soft tumors shows that for the most part the cells are large oval to spindle cells and many contain mitotic figures, *i.e.*, often three or four in an oil immersion field. The protoplasm is finely granular and fairly definite; its limits are sharply marked by numerous myoglia fibrils (See Plate VII., Fig. 1). Cells of this type form the greater part of both tumors, and they stand out in marked contrast to the smooth muscle tissue which they are everywhere invading and destroying. In places the cells of this type grade off into others which are larger, more irregular in shape, often approaching the spherical, and contain large lobulated or multiple nuclei. The myoglia fibrils of these cells are finer and not so well developed as those of the more spindle-shaped cells. So far as mitotic figures are concerned, the cells certainly contain no more than those parts where the type of cell is more regular and the differentiation of fibrils more perfect. In some parts of the tumor the two types of cells are fairly intimately combined.

There is no question but that this tumor is malignant. This malignancy is shown by the rapidity of growth and the invasion of smooth muscle tissue. Whether the rapidly growing tumor started in an old leiomyoma or invaded it from without does not concern us here. The important question is the one of diagnosis. Did the tumor start from smooth muscle cells or from connective tissue cells? This question of the origin and true nature of certain rapidly growing tumors of the uterus is one of the vexed questions in pathology. In the past the attempt has been made to prove that some of these rapidly growing tumors of the uterus arise from smooth muscle cells and are therefore malignant leiomyomata by demonstrating in different parts of the tumor the gradual transformation of smooth muscle cells into tumor cells. It is now pretty generally agreed, however, that there is no continuous transformation of normal cells into tumor cells, but that the tumor grows only by proliferation of its own cells.

If the origin of rapidly growing tumors from smooth muscle cells is to be proved at all it must be by showing that the tumor cells tend to differentiate so as to produce the morphology of smooth muscle cells, and therefore cannot be connective tissue cells and cannot have arisen from them.

Spindle cell sarcomata occur in the uterus, but can be fairly easily distinguished from malignant leiomyomata. The cells are smaller and the protoplasm is poorly defined. In addition the cells produce both fibroglia and intercellular connective tissue fibrils which are very fine and occur evenly distributed around each cell.

So far I have referred only briefly to the intercellular connective tissue fibrils in leiomyomata. These intercellular fibrils vary greatly in number. In some very dense, slow growing tumors they are few in number, while in others they may be numerous. In the rapidly growing tumors also they vary in number, but are usually few. If they were produced by the smooth muscle cells they would occur in equal number around cells growing at the same rate of speed and would be most numerous in those tumors growing at the lowest rates of speed, just as occurs in the series of tumors arising from connective tissue cells, namely, spindle cell sarcoma, fibrosarcoma, and fibroma. This regularity of distribution, however, is not the case in leiomyomata, for intercellular fibrils are sometimes very scarce even in dense, slow growing leiomyomata. Moreover, the fibrils occur irregularly, often in more or less coarse bundles, and form a sort of reticulum which holds the cells together. This is particularly noticeable in the more rapidly growing tumors.

Careful examination shows that these intercellular connective tissue fibrils in leiomyomata are produced by connective tissue cells, which together with blood vessels form the stroma of the tumor. They tend to surround every smooth muscle cell just as in normal smooth muscle tissue. They never surround the muscle cells in such a way as to give rise to an alveolar arrangement, nor do they form the kind of reticulum lined here and there with endothelial cells, such as occurs in

malignant lymphomata. Here and there among them it is possible to demonstrate fibroglia fibrils in small numbers.

The smooth muscle cells are the only essential cells of a leiomyoma; they alone give rise to metastases. The connective tissue cells and the blood vessels of the stroma represent the reaction on the part of the normal tissue to the physiological demand made on them for nutrition and support by the tumor cells. The terms "fibromyoma" and "fibroid" are misleading, and should, therefore, be given up, because they imply that the connective tissue forms an essential part of the tumor when, in fact, it occupies the same secondary position as in a dense or scirrhous carcinoma.

With regard to the leiomyomata it is a little difficult to state just what constitutes malignancy. Some may grow rapidly and yet up to the time of removal give no sign of invading the surrounding tissue or of giving rise to metastases. It is probable, however, that such tumors would betray their malignancy in time but for removal. On the other hand, dense leiomyomata have several times been reported which have given rise to metastases. Whether any of these cases proved fatal is impossible for me to state. Possibly careful examination would have shown the presence of occasional mitotic figures in these tumors pointing to more rapid growth than was suspected.

FIBROGLIA FIBRILS.

Under this heading I consider briefly certain tumors of which the cells and intercellular substances differentiate alike. Of these tumors, at least the fibroma, the fibrosarcoma, and the spindle cell sarcoma should be included in one group and treated as an entity.

I have already shown[1] that connective tissue cells produce, besides the elastic and the ordinary intercellular connective tissue fibrils, another kind of fibril for which the name of fibroglia fibril was proposed. These fibroglia fibrils differ chemically from the intercellular and elastic tissue fibrils, and

[1] Mallory. Journal of Medical Research, 1903, Vol. x, p. 334.

can be stained differentially from them by a variety of staining methods, but most satisfactorily by the methods given in detail in this paper. These fibrils bear much the same relation to connective tissue cells that neuroglia fibrils bear to neuroglia cells, *i.e.*, as stated in the introduction to this paper, they touch or form part of the periphery of the cell protoplasm for a certain distance in the neighborhood of the nucleus and then continue away from the cell in opposite directions. How far they are accompanied by protoplasmic processes cannot be determined. The number of fibroglia fibrils to a cell is not constant, but it is usually in the neighborhood of a dozen.

The typical connective cell is a flattened cell lying in a plane along which it sends out two or more protoplasmic processes. The nucleus is oval, but flattened. The protoplasm is of delicate texture and does not stain at all deeply. The fibroglia fibrils lie in the same plane as the protoplasm and spread out in a more or less fan-shaped manner between layers of the intercellular fibrils. Their course is straight or curved, but rarely wavy.

When seen from the side connective tissue cells are spindle-shaped, and the fibroglia fibrils seem to run in straight lines only.

The typical flattened shape of connective tissue cells and the number, size, and general course of the fibroglia fibrils are best studied in young, actively developing connective tissue, for example in granulation tissue and in the stroma of carcinomata.

Fibroglia fibrils are in general distinctly finer than neuroglia fibrils. In very young connective tissue it is difficult to distinguish them except when seen on end, even when an oil immersion lens and the best illumination are used.

The study of a series of fibromata, fibrosarcomata, and spindle cell sarcomata show much the same condition of the fibroglia fibrils as in normal and inflammatory connective tissue. In the slowest growing tumors the fibrils are few in number. They are best developed and most easily seen in those tumors which are growing at a moderate degree of

speed, *i.e.*, in those tumors which contain only an occasional mitotic figure and which would be classified as fibrosarcomata or as slow growing spindle cell sarcomata. In the most rapidly growing tumors of this series the fibrils are exceedingly fine and require the best fixation, staining, and illumination to demonstrate them clearly.

The ordinary intercellular fibrils produced by the cells are likewise exceedingly delicate in the most cellular tumors, and surround in equal number all those cells which are growing at the same rate of speed. In the more slowly growing tumors the intercellular fibrils become more numerous, while in the fibromata, in contrast to the fibroglia fibrils which are few in number, they form the bulk of the tumor.

The flattened form of the typical connective tissue cell is uniformly preserved in all tumors of this series, except in the most rapidly growing of the spindle cell sarcomata; in them the flattened shape seems to be entirely lost. So far as can be judged from a single case the fibrils in a keloid do not differ from those in a fibroma. In a case of multiple spontaneous keloid [1] the connective tissue cells are in well marked layers between thick, more or less hyaline, layers of intercellular connective tissue fibrils. The fibroglia fibrils are numerous and well developed. In a recurrence after a removal of one of the keloids, the connective tissue cells are in fairly active proliferation and show numerous well formed fibroglia fibrils. The tissue resembles young, actively growing connective tissue of inflammatory origin or a fibrosarcoma.

It would seem as though the fibroma, the fibrosarcoma, and the spindle cell sarcoma should be considered by themselves as an entity, in order that the common characteristics of the tumors in which the cells tend to differentiate like ordinary connective cells may be rendered more prominent. There is of course this difficulty about considering this group of tumors by themselves, that ordinary myxomatous and connective tissues, cartilage, and bone are embryologically so closely related. Moreover, the question of the production

[1] I am indebted for the tissue of this case to Dr. M. F. Austin, pathologist to the New England Hospital for Women and Children.

of a reticulum, chemically as well as morphologically distinct from the ordinary intercellular connective tissue fibrils, is as yet unsettled. Still the attempt should be made to separate those slowly or rapidly growing tumors, the cells of which tend to differentiate like the cells of different normal tissues, into their respective groups, and to treat them as entities. Ribbert has already made a plea for such a separate consideration of those tumors which tend to differentiate like myxomatous tissue.

It is unfortunate that the term "fibrosarcoma" is employed to designate those tumors which are growing more rapidly than the fibroma, but not so rapidly as the spindle cell sarcoma. We have the terms "myxoma," "myxosarcoma;" "chondroma," "chondrosarcoma;" "osteoma," "osteosarcoma" to express the slowest and most rapid rates of growth of these three groups of tumors. It would be simpler and more consistent to use the term "fibrosarcoma" for the most rapidly growing tumors of the nature of connective tissue and to give up the term "spindle cell sarcoma."

My experience with perfectly preserved tissues of all of the tumors enumerated above is too limited to allow any general statements at the present time, but it is doubtful if fibroglia fibrils are produced by cartilage or bone cells. A word may be said, however, in regard to the group of tumors called giant cell sarcomata. Some of these tumors grow rapidly, others very slowly. It is doubtful if all of them should be classed as sarcomata. They seem to stand in close relationship to the tumors of which the cells tend to differentiate like ordinary connective tissue, and should, perhaps, be classed as a variety of them, because the cells other than the giant cells produce both fibroglia and intercellular fibrils.

In the group of tumors of the breast classified as adenoma, adeno-fibroma, and intracanalicular papillary adenofibroma, fibroglia fibrils occur all through the connective tissue part of the tumors. They are scarce in the densest, most slowly growing portions of the tumors, but are abundant wherever the connective tissue is developing. In the more rapidly growing tumors, in which the connective tissue portion

approaches the fibrosarcoma and the spindle cell sarcoma in the relative amount of cell and intercellular substance, the fibroglia fibrils are very numerous. In addition to these fibrils of the connective tissue there is another set of fibrils which occurs beneath the lining epithelium of the glands and to which attention has already been called.[1]

These fibrils vary in number, but are usually abundant. They run lengthwise of the glands and ducts, and belong to cells running in the same direction. These cells with their fibrils lie inside of the hyaline membrane which surrounds the normal glands of the breast, and form the true basement membrane on which the epithelium rests. I have regarded these cells with fibrils as connective tissue cells of a special type, but am unable so far to prove absolutely that they are. Certainly the fibrils do not disintegrate after removal from the body so rapidly as do myoglia fibrils. In the tumors above mentioned these cells with their fibrils always accompany the epithelium of the new formed glands, and seem to form just as essential a part of the tumor as the epithelium.

It cannot be said, however, that the presence of fibroglia fibrils throws any light on the classification of this class of tumors. Whether they should be classed as epithelial tumors, as connective tumors, or as fibro-epithelial tumors is still an open question. If they gave rise to metastases the true tumor cells of the growths could easily be determined.

SUMMARY AND CONCLUSIONS.

Neuroglia, smooth muscle, and connective tissue cells all produce fibrils which morphologically and in certain staining reactions more or less closely resemble one another. These fibrils have been named neuroglia, myoglia, and fibroglia fibrils respectively. They touch or form part of the periphery of the cell protoplasm, but continue away from the cell in two directions, *i.e.*, they do not begin or end in the cell which produces them. Away from the cell to which they owe their existence, neuroglia and fibroglia fibrils seem to be free

[1] Mallory, loc. cit.

of the protoplasm of other cells, but myoglia fibrils seem to run along other muscle cells or to join the fibrils produced by them.

Of these three kinds of fibrils it may be said in general that the fibroglia fibrils are the finest, the myoglia fibrils the most sensitive to post-mortem changes.

In tumors which arise from neuroglia, smooth muscle, or connective tissue cells, the cells tend to differentiate in nucleus, cytoplasm, and intercellular substances in the same way as the corresponding normal cells do, but the differentiation is most complete in those tumors which are growing most slowly. Even in the most rapidly growing tumors, however, the differentiation is usually, perhaps, always far enough advanced to identify the character of the cells, provided the tumor tissue is properly fixed directly after removal by operation.

All tumors, no matter how slowly or how rapidly they are growing, in which the cells tend to differentiate alike in cell and intercellular substance should be included under one heading and treated as a definite entity, in order that the common characteristics may be rendered prominent.

DESCRIPTION OF PLATES.

All the microscopic drawings were made with Zeiss homogeneous oil immersion two millimeters, compensation ocular 6.

PLATE V.

FIGS. 1 and 2. Sections through recurrence of glioma over coccyx.

PLATE VI.

FIG. 1. Neuroglia cells and fibrils in alveolus of glioma over coccyx.

FIG. 2. Connective tissue stroma of glioma over coccyx.

Figs. 1 and 2 stained by Method 4. Neuroglia and fibroglia fibrils blue, intercellular fibrils reddish.

PLATE VII.

FIG. 1. Malignant leiomyoma.

FIG. 2. Leiomyoma.

Figs. 1 and 2 stained by Method 2. Myoglia fibrils red, intercellular connective tissue fibrils pale blue.

PLATE VIII.

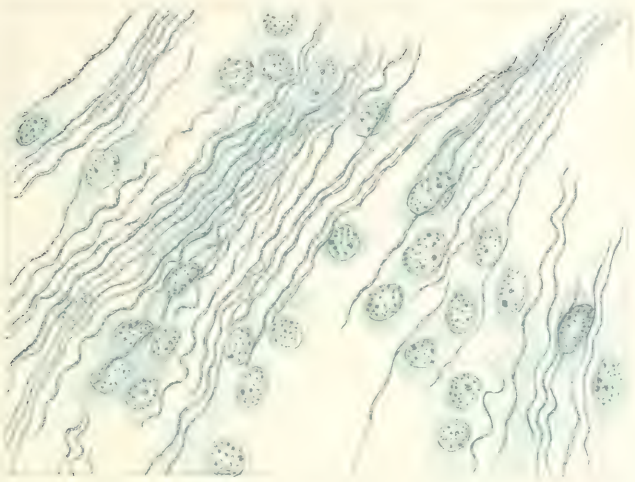
FIG. 1. Stroma of a carcinoma of the breast.

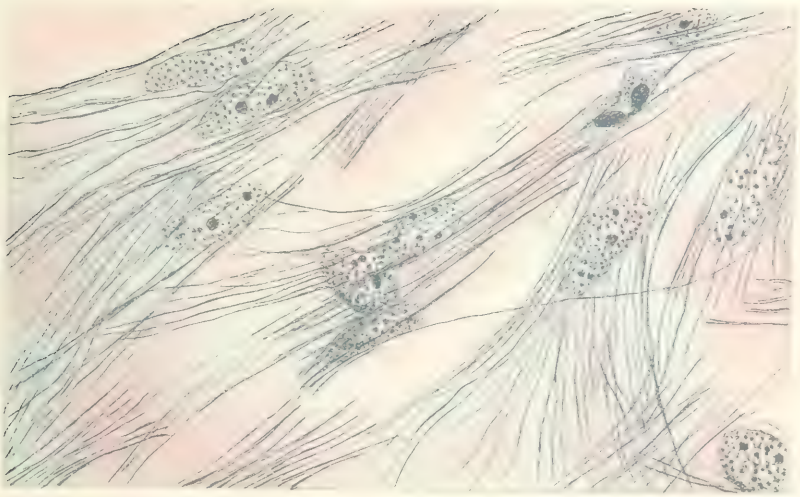
FIG. 2 Recurrent keloid.

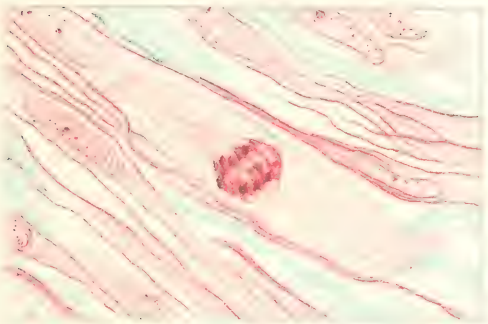
FIGS. 3, 4, and 5. Fairly rapidly growing spindle cell sarcoma. In Fig. 3 the cells are seen flatwise, in Fig. 4 from the side, and in Fig. 5 in cross section.

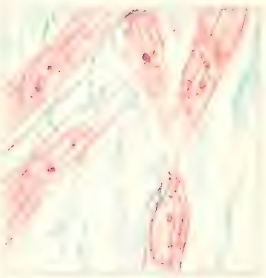
Figs. 1, 2, 3, 4, and 5 stained by Method 2. Fibroglia fibrils red, intercellular fibrils pale blue, elastic tissue yellow.

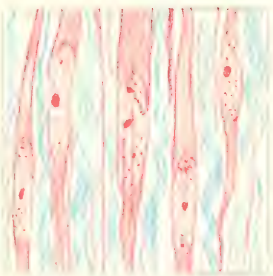
1 and 2.

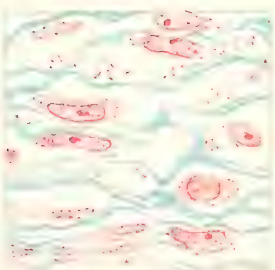












ON THE NATURE OF THE CELL INCLUSIONS OF CANCER.*

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In the first report of the Cancer Investigation Committee of the Harvard Medical School, in 1900,[1] the writer reported the examination of a series of specimens of carcinoma for "Plimmer's bodies." In the second report of the Cancer Investigation Committee, in 1902,[2] a more extensive study of cell inclusions was undertaken, and the nature of these appearances in cancer and in non-cancerous tissue was discussed. The following conclusions were then recorded:

1. Cell inclusions of a constant type are found in practically all cases of cancer of the mammary gland.

2. They are also found in non-cancerous disease of the mammary gland.

3. They are not found in epithelioma or sarcoma.

4. Their appearance, staining reactions, and situation in the cell are such as to justify the hypothesis that they are the result of the secretory activity of the epithelial cell.

5. There is no cause for attributing a parasitic origin to their appearance.

The meeting of the Harvard Cancer Committee, at which Report No. 2 was delivered, was held in February, 1902. The publication of this report was made in the Journal of Medical Research in April, 1902, and in the Bulletin of the Cancer Committee which was issued as a reprint of this publication. In April, 1902, Dr. Hans Nösske,[3] from Professor Marchaud's laboratory, Leipzig, read a communication to the Deutsche Gesellschaft für Chirurgie, which was published in brief in the proceedings of that society, 1902 meeting, No. 31, ii, p. 305, and at greater length in the Deutsche Zeitschrift für Chirurgie, July, 1902, lxiv,

* Received for publication Nov. 1, 1904.

352. In this report, as the result of the examination of a large series of specimens, Nösske arrived at the following conclusions (translation): "That the Plimmer's body is nothing more than a specially characteristic vacuole, filled with a coagulable substance very varied in concentration and quantity, which occurs chiefly in the protoplasm of the cells of gland cancer, but occasionally also in the proliferating gland cells of benign tumors and in inflammatory processes." These structures Nösske regards as "specially formed vacuoles produced by a kind of internal secretion in the protoplasm of the cells of gland cancer," and the facts upon which this conclusion is based are much the same as those detailed in my own report, No. 2; that is, the presence of inclusions in gland cancer and in non-cancerous gland tissues and their absence in sarcoma and epithelioma. A number of other papers which have appeared since April, 1902, have afforded further confirmation of these observations, and have aided to establish the secretion hypothesis upon a more secure foundation. Among these articles must be mentioned those of Klimenko,[4] Spirlas,[5] Apolant and Embden,[6] and especially that of Honda,[7] of Hansemann's laboratory. Honda's conclusions, as the result of the examination of a large series of specimens, are as follows (translation):

"1. The characteristic forms of cell inclusions, at present under discussion, of Plimmer (Gaylord), Feinberg, and apparently also of von Leyden, are not at all, as believed by these writers, of different nature. They are all similar forms which are merely made evident by different methods.

"2. These structures are met with only in a special type of cancer — gland cancer. I have found them, however, in other neoplasms and inflammatory processes, although more rarely and fewer in number. This is evidence against the supposition that they are of a parasitic nature. A further argument against such a supposition is the lack of correlation between the comparatively rare occurrence of the bodies in question and the greatly increased proliferative ability of cancer cells.

"3. The nature of the bodies in question, of the above-named writers, cannot be explained with certainty at the present time, although they should certainly not be regarded as cancer parasites. They are apparently produced by the secretion of hyaline material in the cell protoplasm, or also by a partial hyaline degeneration of the cell protoplasm."

In the present communication it is the purpose to record the results of further observations, to assemble some of the observations of others, and to review the secretion hypothesis as it stands at the present time.

The material for this investigation has been supplied through the kindness of Dr. W. F. Whitney and Dr. J. H. Wright, of the Massachusetts General Hospital, and for these and many other favors the writer wishes to express to them his deepest obligations. The technic employed in fixing and hardening tissues, embedding specimens, and making and staining sections was the same as that detailed in previous reports (Nos. 1 and 2). In the main, Zenker's fluid as a fixative, and iron hematoxylon (Mallory) and orange G. and fuchsin as a routine double stain were the methods which gave most satisfactory results.

The material forming the basis of this report comprises sixty-three specimens of tissue. Fifty-seven of these specimens were obtained after the publication of Report No. 2, and six specimens which had already been described in Reports No. 1 and No. 2 were included in this series for comparison.

The description of cell inclusions or "cancer bodies," which has served as a criterion for the determination of their presence or absence in a given tissue, is that published by Plimmer[8] in his oft-quoted article in the Practitioner of April, 1899. It has been quoted at length in Report No. 2, but for convenience is again repeated.

"The parasites, as they most often occur, are round bodies of very diverse sizes, from .004 millimeter to .04 millimeter, or even more, in diameter. There is a central portion, which I shall call here, for convenience, the nucleus, although there is nothing in this central portion in common with

the biological nucleus, which is generally round, but which may be irregular in shape. Around the nucleus is a layer of protoplasm, and outside this is a capsule. This nucleus differs in its micro-chemical reactions from the nucleus of the cell. It takes, with the Ehrlich-Biondi solution, a copper-red color; with thionin, a dark purple; and with (1) of the double hematoxylon stains, described on page 440, it also takes a copper-red color quite different from the red of either the protoplasm or the fibrous stroma, and with (2) it takes a dark claret color again darker than that of the protoplasm or stroma. With the Ehrlich-Biondi stain it reacts somewhat like the nucleolus of the cancer-cell nucleus, but the color of the latter is much brighter; moreover, with many other stains — such as anilin blue or *bleu de Lyon* or anilin green — it does not, as does the nucleolus, lose the stain when washed with spirit, but retains it even after prolonged immersion. This is to be remembered against the assertion that these bodies are aberrant nuclear structures. In very perfectly fixed specimens a lighter spot can be observed in the nucleus, which is not visible in fresh specimens, but in fresh specimens the nucleus appears to be larger than in fixed ones. It is to be noted that the nucleus is practically refractory to hematoxylon used in the strongest manner possible, when this is done by the ordinary methods. This, again, is very important in connection with the statement that the bodies are nuclear structures. With fixation in alcohol, which is of no use as a fine fixative, and the use of certain stains, such as the Ehrlich-Biondi mixture, a marked metachromatism is seen — the nucleus of the parasite being violet, and that of the cell blue-green. . . .

"The layer of protoplasm around the nucleus is generally homogeneous, and stains much less deeply than the nucleus. With alcohol fixation, again, the phenomenon of metachromatism is sometimes seen, the protoplasm staining blue with the Ehrlich-Biondi mixture. When the parasite has attained a certain size a rayed appearance is often seen in the protoplasm, but I have never seen this in the fresh state, and think it may be due to the fixative.

"The capsule is a very well-marked structure, which can be seen well both in the fresh as well as in fixed specimens. That this capsule is a part of the parasite can be demonstrated by the fact that it can sometimes be seen folded over on itself and sometimes from fixation it shrinks away from the protoplasm of the cell, and also the parasite can sometimes be seen free in an alveolus with its capsule around it. It stains more definitely than either the nucleus or protoplasm. With the Ehrlich-Biondi mixture it is a brighter red than either of the other structures; with thionin it is darker; with hematoxylon, acid fuchsin, and orange it is a clear bright red, and with hematoxylon and Bordeaux red it shows best as a very bright red line.

"This description applies to the forms most commonly met with, but there are some other forms which are met with, especially in cancers of rapid growth. They are, however, rare, so I will merely tabulate them." . . .

A significant result of the investigation published by the writer in 1902 [2] was the occurrence of cell inclusions in specimens of chronic cystic mastitis, in which no evidence of cancer could be detected. A further study of material of this nature was made possible by a collection of cases of chronic cystic mastitis which was made for another purpose, and the study of which was reported in 1903 (Greenough and Hartwell [10]). Twenty-three cases of mastitis were thus available which had not been included in the 1902 report. In sixteen cases typical inclusions were found, and in seven cases they could not be detected. The degree of epithelial proliferation in chronic cystic mastitis was found, in the study to which reference has been made, to exhibit marked variation in extent. Four varieties of epithelial proliferation were there distinguished — acinal, columnar, papillary, and "adenomatous" growth. Of the twenty-three cases of chronic cystic mastitis twenty showed columnar proliferation; eleven of these cases with columnar growth presented papillary outgrowths, and five of the cases with papillary outgrowth showed also "adenomatous proliferation." It is of interest to note that of the five cases of adenomatous growth all presented typical cell inclusions. Of the eleven cases of papillary growth ten showed inclusions, and of the twenty cases with columnar cells fifteen showed cell inclusions. There were three cases of acinal proliferation without columnar or papillary growth, and in two of the three inclusions could be demonstrated. The cell inclusions in chronic cystic mastitis answer in every way to Plimmer's classical description. The staining reactions and the situation in the cell were typical, and the size of the inclusions corresponded with Plimmer's figures, but the larger forms, which are found in cancer in large numbers, were generally absent (Plate X., Fig. 13.) The presence of cell inclusions in chronic cystic mastitis, as in other lesions of the breast, appears to depend more upon the size and degree of proliferation of the epithelium than upon any other factor. In the seven cases in which no inclusions were to be detected there were either marked degenerative changes in the tissue, the fixation was

imperfect (as with alcohol-hardened specimens), or the cells were small and showed little evidence of proliferative activity. The presence of inclusions in all of the cases of adenomatous proliferation, and in eleven out of twelve cases of papillary growth, is a concrete example of the constancy of the occurrence of inclusions in proliferated breast epithelium. The situation in the cell of the inclusions in these cases was also in accord with previous observations. In columnar and papillary types of epithelial growth the inclusions were situated invariably toward the free border of the cell, on the side of the nucleus, away from the basement membrane, and toward the lumen of the duct or cyst. In cases of adenomatous proliferation, where new gland lumina are formed by the coalescence of papillary outgrowths of epithelium, the bodies in the same manner lay on the lumen side of the nucleus. In addition to the typical inclusions with central protoplasmic-staining body, there were, moreover, many similar *empty* vacuoles. These were of such a size and shape as to confirm the observations previously made that vacuolar structures of this nature are essentially the same as the so-called typical cell inclusion, and that they lack only the coagulated and contracted contents which is the mark of identification of the " Plimmer's body."

Thirteen other specimens of non-cancerous diseases of the female breast were examined during the year. Four cases of adeno-fibroma were available for examination, and of this number three showed typical inclusions in the epithelial cells on the side of the nucleus toward the lumen of the gland (Plate X. and Figs. 11–12). Four cases of cyst-adenoma of the breast showed inclusions in two instances in a similar position with relation to the lumen of the duct. In another specimen of cyst-adenoma of the breast which was reported in 1902, but of which new preparations were made, epithelial proliferation of a marked degree was present, and numerous inclusions were found in the familiar situation between the nucleus and the gland lumen (Plate X., Fig. 9). The breast of a woman in the third month of pregnancy and that of

another woman shortly after labor showed many vacuolar structures in the cell protoplasm. In some of these vacuoles a central body was present, and the typical cell inclusion could be identified. In two normal breasts, however, and in another case of chronic suppurative mastitis no inclusions could be found.

Eight new cases of breast cancer were examined, and five cases previously reported were subjected to further examination. In all but one of the eight new specimens inclusions were readily demonstrated. In that one, a very advanced case of general carcinomatosis originating in the breast, the cells were unusually small and showed scarcely more protoplasm than a lymphocyte; no intracellular structures could be found, and it was considered probable that the absence of cell inclusions was due to the extreme departure of the cells from the normal gland type by which they had lost entirely their previous ability to produce secretion. The consideration of the appearance and situation of the inclusions in the cases of cancer of the breast will be taken up in detail in a later paragraph. It is sufficient to say that in all, with the exception of the one case above detailed, inclusions were present of variable size and of a typical character, and that vacuolar structures of exactly similar appearance, lacking only the central body, were also present in considerable numbers in every case.

A number of specimens of adenoma and of cancer of glandular organs other than the breast were examined. Of these tissues an adenoma of the thyroid, with proliferated epithelium and solid columns of cells, showed typical inclusions of the smaller varieties, not exceeding in size one-half the diameter of a red blood corpuscle. In three specimens of adenomata from the ovary, the pancreas, and the prostate inclusions could not be found. Evidence of secretion was present in the form of granules in the cell protoplasm in that portion of the cell nearest to the gland lumen, and large vacuoles were present, but no central body could be detected in the smaller vacuoles.

A case of carcinoma of the stomach in good preservation showed typical inclusions, and a case of metastatic carcinoma of the liver, the primary origin of which could not be determined, showed also the typical appearance of cancer inclusions in the cells A case of cancer of the prostate showed inclusions in a few cells with sharp circular outline, faint periphery, and dark central staining area. As in the thyroid, the inclusions were of small size, and the amount of central staining material varied more than is usual in breast cancer. Six cases of carcinoma of the rectum were obtained, and in five cases inclusions were present. They could not be found in one case in which the fixation was unsatisfactory and post-mortem degeneration had set in. In cancer of the rectum again the cell inclusions differed slightly from those found in the breast. They were, as a rule, larger and contained less central-staining material in proportion to their size. In adeno-carcinoma types of cancer of the rectum the inclusions were found on the lumen side of the nucleus. The presence of basic-staining material in the central bodies of the inclusions was notable in cancer of the rectum, but whether due to chromatin or to mucin in the material could not be determined. The comparatively large amount of basic-staining material, however, made it appear that a mucinous composition was the more probable constituent.

The pathological material examined for cell inclusions by the writer since 1899 comprises in all one hundred and seventy-seven different specimens of tissue. Twenty-eight were reported in 1900, ninety-two in 1902, and fifty-seven in the present paper. A tabulation of these specimens, together with the observations reported by Nichols,[1] Nösske,[3] and Honda,[7] provides data upon a total of four hundred and eighty-one specimens. This number, while not by any means so large as Plimmer's report of 1,278 cases,* is still great enough

* "During the past six years I have examined microscopically 1,278 cancers, taken as they came from many parts and organs, and of all possible varieties. I have, on account of the necessity of verbal accuracy in regard to the title of this paper, omitted sarcomata, and the analysis given below refers exclusively to carcinomata.

to neutralize individual errors of technic and of observation, and forms the only large collection of observations upon the presence or absence of cell inclusions in cancer and other tissues which is at present available for comparison with Plimmer's work.

Analysis of 1,278 cases of cancer, arranged in numerical order according to locality:

Locality.	Primary.	Secondary and Recurrent.
Breast	410	113
Skin, including tongue and penis	368	38
Uterus and vagina	105	1
Stomach and intestines	46	5
Liver	..	24
Pancreas	1	..
Lung	..	5
Bladder	3	..
Glands	..	160
	936	342

I have found the parasitic bodies in 1,130 out of this total of 1,278 cases. Of those in which I failed to find them, sixty-three were densely fibrous and atrophic — in fact, cured locally; twenty-seven were so degenerated that with very careful examination no stain could be made to affect the broken up and dead cells. The parasites are not found in all parts of the cancer; they are only found for certain at the growing edge. I have failed to find them in the degenerated parts, or in those parts where the fibrous tissue is destroying or digesting the cancer cells. The same statement applies to the metastases, so that these bodies are only found in active cells and not in those which show retrograde or degenerative changes. They may be seen free in an alveolus still surrounded with their capsule, and having the same reactions which they have in the cells."— *Plimmer, Practitioner, April, 1899.*

TABLE OF SPECIMENS EXAMINED FOR "CANCER BODIES."

	Greenough.		Nosske.		Nichols.		Honda.		Total.	
	No.	Present.	No.	Present.	No.	Present.	No.	Present.	No.	Present.
Carcinoma, 118 cases, 185 examined—154 positive—83 per cent.										
Breast	50	38	25	25	10	13	32	24	117	100
Stomach	1	1	5	5	.....	.....	12	8	18	14
Rectum	0	8	0	3	.....	.....	2	1	11	12
Intestine	.....	.....	1	1	1	1	3	3	5	5
Ovary	2	2	2	2	.....	.....	2	2	6	6
Gall-bladder	.....	.....	4	4	.....	.....	3	2	7	6
Breast	.....	.....	3	3	.....	.....	1	0	4	3
Bladder	.....	.....	2	1	1	0	1	0	4	1
Other glands	2	2	7	5	3	2	3	1	15	10
									185	154
Sarcoma, 32 cases—1 positive—3 per cent.	3	—	8	0	5	0	14	1	30	1
Suprarenal tumors, 5 cases—4 positive—80 per cent.	.....	.....	2	2	.....	.....	3	2	5	4
Epithelioma, 27 cases—none positive—0 per cent.										
Fibro-cellular	10	—	5	0	8	0	1	0	27	0

Tongue			3	0			1		4	0
Penis					3	0			3	0
Vulva, vagina, cervix			6	0	1	0	0	0	13	0
Paget's disease of breast	1	0							1	0
Elsewhere	7	0	18	0	2	0	1	0	31	0
									79	0
Adenoma, 15 cases — 2 positive 13 per cent.										
Ovary	5	1							5	1
Thyroid	5	1					1	0	6	1
Prostate	1	0							1	0
Pancreas	1	0							1	0
Stomach (polyp)	0	0					1	0	1	0
Lung (guinea pig)							1	0	1	0
									15	2
Non-cancerous diseases of breast, 72 cases — 49 positive — 68 %.										
Adeno fibroma	12	8							12	8
Cyst adenoma	8	5	3	3					11	8
Chronic cystic mastitis	34	25	1	1					35	26
Other lesions	0	7	2	0			3	0	14	11
									72	53

TABLE OF SPECIMENS EXAMINED FOR "CANCER BODIES." — *Continued.*

	Greenough.		Nisske.		Nichols.		Honda.		Total.	
	No.	Present.	No.	Present.	No.	Present.	No.	Present.	No.	Present.
Miscellaneous cases (7 positive)										
Normal tissues	14	0	2	0			8	0	22	0
Animal tissues	7	0					1	0	8	0
Non-cancerous cases; of bladder							7	0	7	0
Of lung	2	0	1	1			8	5	11	6
Of liver							5	1	5	1
Of spleen							1	0	1	0
Of kidney							1	0	1	0
Of lymph glands	1	0							1	0
Papilloma of skin	2	0					2	0	4	0
Scarified rabbit ears							11	0	11	0
Dermoid cysts	1	0					1	0	2	0
Deciduoma malignum							1	0	1	0
Tuberculosis	4	0					2	0	6	0
Syphilis							2	0	2	0
Actinomycosis							1	0	1	0
Miscellaneous							12	0	12	0
Total	177	94	110	57	10	10	158	50	481	217

In this collection of four hundred and eighty-one cases the most conspicuous fact is the inconstant occurrence of cell inclusions in the different varieties of malignant disease. In cancer of glandular organs inclusions are to be found in nearly every case. Of one hundred and eighty-five cases of cancer of gland origin, one hundred and fifty-four showed typical inclusions or eighty-three per cent. In mammary cancer the constancy of their occurrence is even greater; of one hundred and twelve cases, one hundred gave positive results or eighty-nine per cent. This fact, however, is not surprising when it is remembered that breast cancer has long been the favorite material for the demonstration of cell inclusions, and a glance at the plates of the many writers on the parasitic etiology of cancer will prove that the source of their most suggestive pictures was almost invariably a specimen of cancer of glandular origin. The protozoan forms described by Thoma, in 1889,[24] were found in cancer of the large intestine, the stomach, and the breast, and subsequent writers have been unable to extend the field of profitable search to any great extent. As a fact, it appears almost as if the failure to detect cell inclusions in gland cancer could be attributed either to defective technic of preparation or to defective preservation of the tissues.

It will be noted that cancer of the bladder has been included among the cases of cancer of glandular origin. The bladder contains squamous cells, and might be supposed to produce epithelioma. As a fact, however, the epithelium of the bladder is derived from the entoderm of the allantois (Minot[27]), and cancer of the bladder is of the gland type and does not resemble squamous celled cancer or epithelioma in any way. Whether the bladder epithelium is normally endowed with secretory function is at present a disputed point in histology (Böhm, Davidoff, Hüber,[26] Kœlliker[25]), but that this function is rapidly acquired in disease is readily proved to the clinician by the abundant ropy mucus, the secretion of which is so marked a phenomenon in tumors and diseases of this organ. The occurrence of cell inclusions in bladder cancer has long been a well-established fact

(Hansemann,[18] page 111), of which the positive report of Nösske only offers confirmation.

In thirty cases of sarcoma of many different varieties, inclusions were found in but a single case, by Honda. No explanation is offered for this single positive observation, and indeed the variety of sarcoma is not stated. As it is, the observation must be allowed to stand as the exception which proves the rule that cell inclusions of the Plimmer's body or bird's-eye type are not found in sarcoma. Further reference to this observation will be made, however, in connection with the occurrence of nuclear degenerations in cancer and sarcoma in a later paragraph.

Of five cases of perithelioma or hypernephroma, inclusions were found to be present in four specimens by Honda and by Nösske. It is to be remembered that the cells of the suprarenal tumors show a notorious tendency to fatty degeneration and vacuolization. In the later discussion of the nature of cell inclusions the significance of these phenomena will be apparent.

Seventy-nine specimens of epithelioma are included in the table with the astonishing result that typical inclusions of the type of Plimmer's bodies were not found in a single case. The specimens included squamous cell carcinoma of the skin of the face, the lip, tongue, penis, vulva, cervix, and other regions. In two specimens examined by the writer vacuolar structures were found, which were interpreted as degenerative changes and were classed as atypical inclusions, and in one case examined by Nichols inclusions were found in the normal uterine mucosa beyond the margins of the malignant growth, although not present in the cancer tissue. These results are thus directly contradictory to those of Plimmer. Plimmer's later contention,[9] that the failure to discover inclusions in epithelioma is due to a failure to persist for a sufficient time in the search for them, cannot be taken seriously. Prolonged search must indeed be needed if the difficulties in their discovery are so very great that in seventy-nine cases carefully examined by competent observers familiar with the appearance of cell inclusions, not one

inclusion could be found that would pass muster as a so-called Plimmer's body. Another fact, noted originally by Nösske,[3] is that Gaylord,[12] who claims that "the results of our observations completely substantiate Plimmer's claim that these bodies are present in all carcinomata," records "typical Plimmer's bodies, internuclear protozoan forms, and Russell's bodies" as present in forty-four out of forty-five cases of cancer of glandular organs, while "fourteen cases of squamous epithelioma were examined and all of these contained *atypical bodies which gave the staining reactions of Plimmer's bodies, but were much larger and did not contain the characteristic central bodies*," and "fifteen cases of sarcoma, *all of which contained the small forms of the organism within the nuclei. Plimmer's bodies are rare, and not so well defined as in carcinoma.*"

The remaining specimens included in the table are specimens of non-cancerous tissue from a variety of sources,—normal animal tissues and the results of animal experimentation, normal human tissues and a variety of pathological lesions of other than cancerous disease. Fifteen cases of adenoma of glandular organs other than the breast were examined, but with practically negative results, inclusions being found in only two cases in the cells of a papillary cyst-adenoma of the ovary of marked proliferative activity and in an adenoma of the thyroid.

Seventy-two cases of non-malignant breast diseases appear in the table, with the positive discovery of cell inclusions in forty-nine cases. Specimens of adeno-fibroma, cyst-adenoma, chronic cystic mastitis, and other lesions are included in this category. Specimens of cyst-adenoma of the breast appear to be especially rich in cell inclusions, and in general the occurrence of inclusions appears to depend to a great extent upon the degree of proliferation of the glandular epithelium. It is to be noted that inclusions were found by Gaylord[12] in both breasts in a case of multiple adeno-fibroma, and that Klimenko[4] also reports their occurrence in another tumor of this nature.

Of the other ninety-five specimens of non-cancerous tissues

included in the table, the presence of inclusions has been demonstrated in seven cases. They were found by Nösske and by Honda in the bronchial epithelium in six specimens of pulmonary lesions and by Honda in one case of acute yellow atrophy of the liver.

The results of the examination of four hundred and eighty-one specimens of cancerous and other tissues may then be summarized as follows:

1. Cell inclusions are almost constantly present in cancer of glandular origin.

2. They are almost invariably absent in sarcoma.

3. They do not occur in epithelioma (cancer of squamous-celled origin).

4. They are found in hypernephroma.

5. They are found in non-cancerous lesions of the mammary gland.

6. They are found occasionally in other gland epithelium in non-cancerous conditions.

The morphological characteristics of the cell inclusions of cancer have been repeatedly described. For the purpose of this investigation Plimmer's description was accepted. It is obvious, however, that the structures described and figured by Plimmer have been seen and described by a great number of observers, not only since the publication of Plimmer's paper, but before that time. A review of the literature by Gaylord in the Fourth Annual Report of the Cancer Laboratory of the New York State Board of Health makes evident the fact that the typical inclusions of cancer have been figured by many writers, and that although slight differences of form and structure are occasionally detected, the morphological entity of the cancer cell inclusion is thoroughly recognized. Honda's observations[7] have further established the identity of the so-called Plimmer's body with the parasites of Feinberg[14] and with the bird's-eye inclusions of von Leyden.[15] It is sufficient, therefore, for us to confine ourselves to Plimmer's description, and we may feel that what applies to Plimmer's bodies applies also to the reputed parasites of other writers upon the parasitic origin of cancer.

So many theories have been advanced in explanation of the occurrence of cell inclusions in cancer that a consideration of them all within the limits of this communication is impossible. It is sufficient to mention only the more important ones, and of these the most significant is that which attributes the inclusions to a parasite. Every writer upon the parasitic origin of cancer draws his inspirations from the inclusions which are so readily to be discovered in cancer of glandular organs. The identification of the parasite varies with different writers from yeasts and blastomyces to one or another of the many forms of protozoa, but the inclusions of gland cancer are the appearances upon which each of these attempts at identification are ultimately based.

The opponents of the parasitic theory of cancer have also attributed the inclusions to a number of different sources. Not all of these theories merit equal consideration, but the list given by Hansemann[19] may well be repeated. These are: (1) Degenerations of cells and anomalous secretion; (2) phagocytosis; (3) invagination; (4) abortive and pathological mitoses; (5) special organs of the cell archoplasm or paranucleus; (6) extracellular hyaline drops (Russell's bodies); (7) cancer cells themselves. There is good reason to suppose that most of these sources may at one time or another be responsible for cell inclusions in cancer tissues. It is equally certain that no one cause is sufficient to explain all of the rounded intracellular structures with a central body which are found in cancerous and in non-cancerous tissues. Nuclear degenerations, chromatolysis, abnormal and abortive mitoses, and pathological conditions of the nucleolous may undoubtedly produce appearances closely simulating the typical inclusion of cancer cells. Pianese[16] was the first, and Apolant and Embden[6] are the most recent writers who have urged a nuclear origin for cell inclusions. This view is largely based upon the occurrence of chromatin in the inclusions, an observation which had been repeatedly confirmed, but which is open to other interpretation than that the inclusions are degenerate or

abnormal nuclei. This subject will come up for further discussion in a later paragraph.

Borrel's theory,[20] which has been supported by Le Count,[21] attributes the cell inclusions of cancer to a metamorphosis or abnormality of the archoplasm, a portion of the cell protoplasm in which the centrosome is situated, and which is formed or becomes differentiated from the other portions of the cell in the process of mitotic division of the nuclei. The archoplasm is thus essentially a phenomenon of mitotic cell division. Benda[22] claims to have refuted Borrel's theory by the discovery of intact centrosomes in cancer cells in which inclusions were also present. The importance of this observation makes further confirmation necessary. Benda used new and improved methods in the preparation of his specimens, the details of which were not recorded. An article by Galeotti and Lustig,[28] in 1893, records numerous observations of centrosomes in cancer cells, but their plates show no evidence of inclusions. In resting cells a single centrosome was found lying in a pocket in the margin of the nucleus, and in cells approaching mitosis two centrosomes were found at opposite sides of the nucleus, as in the familiar phenomenon of mitotic cell division. The positive observations upon centrosomes in human tissues are, at the present time, so few as to allow of only provisional deductions with regard to their function or their occurrence. Even the number of centrosomes in each individual cell appears to be subject to certain variations. As many as twenty centrosomes have been seen by Hansemann[18] in the multi-polar mitoses of cancer cells. Under these circumstances the reported observations of Benda must await further confirmation, and the occurrence of centrosomes in cancer tissues and in other cells must be the subject of further investigation before Borrel's theory can be so peremptorily demolished.

The more recent publication of Spirlas,[5] of Gœttingen, records the artificial production of bird's-eye inclusions in the lymphocytes of the guinea-pig, by the injection of foreign cells and substances into the peritoneal cavity. These

experiments are at first suggestive of a possible phagocytic action on the part of cancer cells. As a fact, however, it is apparent that in these experiments the albuminous materials were taken up by the lymphocytes, and that the subsequent fixation and staining of the specimens produced coagulation and shrinkage of the fluid contents of the vacuole. This phenomenon is not infrequently observed when vacuoles are present in the cell protoplasm containing fluid material. Aschoff,[23] under whose directions Spirlas' experiments were undertaken, announced before the German Pathological Society, in 1903, that although bird's-eye inclusions could be produced in this manner by phagocytosis, this did not prove that the inclusions were not due to other processes, such as the formation of secretion vacuoles or the vacuolization of the archoplasm.

That a certain proportion of the cell inclusions of cancer are attributable to the abnormal secretion of cancer epithelium is a theory which has been alluded to by many writers. Pianese,[16] in 1896, gave special attention to this hypothesis, and made a study of the phenomena of secretion in normal glandular tissues and in gland cancer. His conclusions upon this subject were as follows (translation): "These special degenerations (of the protoplasm of the cells of glandular carcinoma) are of special interest, in that they are the explanation for the greater number of the supposed cancer parasites of the Thoma-Sjöbring type. I say the greatest number, for it would be very difficult, indeed, by any single process of degeneration, either of the protoplasm alone or of the nucleus alone, to provide an explanation for all of the different parasite forms of this nature which have been described by Soudakewitch, Foà, Podwyssozki, Sawtschenko, Ruffer, Plimmer, Walker, and many others." . . . "I desire here to make only brief reference to the question of the secretion of cancer cells in order to call the reader's attention to the fact that the special forms of degeneration which I am about to describe, and which one finds especially in the protoplasm of gland cancer (forming only the smallest portion ('kleinsten Teil') of the cell inclusions which

have been regarded as parasites), are chiefly special metamorphoses of the cell secretion, and to a less extent special degenerations of the cell protoplasm itself."

Pianese studied cancer tissues by a most elaborate system of histological technique, and endeavored to obtain a differential stain for every possible degenerative material or cell product. The presence of material in the majority of the cell inclusions of cancer, however, which gave the staining reactions of chromatin led Pianese to consider the greater number of these inclusions to be of nuclear origin (Plate IV., Figs. 1 and 2, and Plate V., Fig. 4, Pianese[16]).

From Pianese's publication in 1896 until 1902 the secretion hypothesis, though frequently alluded to, received little particular attention. In 1902 my own publication[2] and that of Nösske[3] appeared upon this subject, and later contributions by Honda[7] and others have contributed to its support.

The characteristics of the cell inclusions of cancer may best be considered under the following subdivisions: (1) occurrence; (2) form; (3) size; (4) staining characteristics; (5) situation in the cell; (6) relation to mitosis and to cell proliferation.

1. *Occurrence:* The occurrence of cell inclusions in cancer and in non-cancerous tissues has been considered at length in the first portion of this communication. It was there made evident that inclusions were especially numerous in cancer of glandular origin, and were present in only the rarest instances in sarcoma or epithelioma. It was also demonstrated that inclusions were found in non-cancerous tissues of glandular organs. The significance of these observations in disproving the parasitic origin of inclusions is so obvious that little need be said. A supposed parasite of cancer which is not found in all forms of cancer and which does occur in other non-cancerous tissues has little in its favor. The relation of inclusions to the epithelial cells of glandular organs, however, becomes conspicuous, and the phenomenon which is shared by the epithelial cells of all glands is the phenomenon of secretion.

2. *Form:* In Plimmer's description the form of cancer cell inclusions is given as round. His plates, however, present a number of oval forms as typical inclusions, and round or oval forms are commonly accepted by other writers. The central body (Central-körperchen), according to Plimmer, is round or irregular in shape. The irregularity of the central body is indeed conspicuous, and almost any fancied form may be discovered in a specimen rich in inclusions if sufficient search is made. The forms described by Gaylord,[13] as evidence of nuclear division of the inclusions, represent only a few of the many modifications of form which may be discovered. Single rounded or angular forms, — rods, — trefoil and heart shapes are not uncommon, and double, triple, and multiple central bodies of equal or unequal size are found. The size of the central body also is subject to great variation, and from a minute spot in a central or excentric position up to an indefinite mass nearly filling out the open space all degrees of transition may be found. The " rayed periphery," although not described as an essential characteristic in Plimmer's definition, is conspicuous in many of his plates, and is present in many well-developed specimens. The nature of the rayed periphery has been the subject of some discussion. Its essential similarity to the appearances of mucin threads in the vacuoles of intestinal epithelium justifies the assumption that it is produced in a similar manner by the coagulation or " centripetalization " (Pianese,[16] Greenough[2]) of the albuminoid materials which in life filled out the vacuolar space in the cell protoplasm, but in fixed specimens have been coagulated and drawn away from the periphery. Plimmer himself states that he is in doubt whether the rayed periphery may not be an artefact. A central body is an essential part of the typical cancer inclusion. It cannot be denied, however, as emphasized by Honda[7] and by Apolant and Embden,[6] that atypical forms resembling the cancer inclusion in every way, save only that they lack the central body, are present in every case in which inclusions are abundant (Fig. 25). In larger forms the absence of central body may indeed be explained by the line of section of the specimen, but in the smaller ones

this explanation is inadequate. It is to be noted, however, that in the absence of a central body a cell inclusion differs in no way from an ordinary vacuole.

3. *Size:* The size of cancer cell inclusions varies within wide limits. The smallest ones, which can be identified as typical forms, resemble so closely minute vacuoles in the protoplasm as to be distinguished with difficulty and only on the identification of the central body. At the other extreme typical forms exceeding the nucleus in size are not uncommon, and larger atypical forms distending the cell and distorting the nucleus into a thin layer occur in close relation with the more typical inclusions (Plate X., Figs. 18–25). Indeed, we are forced to the conclusion that the typical inclusion is only one stage of a structure of much variety of size, the essential nature of which is a vacuole in the cell protoplasm containing a greater or less amount of coagulated material within its borders in the form of a central body and occasionally a "rayed periphery." In non-cancerous gland tissues the inclusions which have been found by the writer and by Nichols,[1] Nösske,[2] Honda[7] and others are of a size comparable rather to the smaller inclusions of gland cancer. The inclusions in adeno-carcinoma are also smaller than those in more advanced cancer. This may be attributed to the fact that the products of secretion of glandular epithelium are normally expelled into the gland lumen upon attaining a definite degree of development. In pathological conditions where the gland lumen is obstructed, and especially in advanced cancer where the cells no longer show a tendency to the formation of a gland lumen but grow in solid columns, one cell against another, no avenue of escape is provided for the products of the cell's secreting activity, and the retained secretion remains within the cell to undergo further growth or to suffer the same processes of degeneration and disintegration to which the cell itself is subject.

4. *Staining characteristics:* The staining characteristics of cancer cell inclusions have been the field for diligent experiment and observation. A differential stain, however, which would serve to distinguish readily and constantly

between the inclusions and other cell products has not yet been achieved. As a rule, protoplasmic or acid stains serve best for the study of inclusions when used in combination with a basic stain to differentiate the nuclei. Plimmer's or Mallory's iron hematoxylon, followed by orange G and acid fuchsin, or by Bordeaux red, are the stains best adapted to their study. They may be found, however, by almost any method of staining which is in use in ordinary pathological technic. It may be said, however, that the Romanowsky (Wright) blood stain, as used by Feinberg,[14] has proved of little value in the experience of the writer. Inclusions can indeed be demonstrated by this stain, but the staining characteristics are so inconstant as to allow little dependence to be put upon their interpretation.

The cancer cell inclusion, when stained by iron hematoxylon and counter-stained with orange G and acid fuchsin, presents the following characteristics: The central body is stained reddish brown in varying shades of color. The smaller the body, the deeper the shade of color, as a rule. Within the central body in many cases, but not by any means in every case, one or several minute black spots are found which have retained the basic hematoxylon stain after differentiation, and must be regarded as chromatin. Surrounding the central body a clear space occurs; this space may be colorless or may present a tinge of protoplasmic stain from the overlying protoplasm. Beyond the open space, in many cases, another area of protoplasmic staining material occurs — the "rayed periphery." This layer is subject to considerable variation, and in some specimens presents almost the appearance of a double contour which has been interpreted as a capsule. As a rule, however, the "rayed periphery" consists of a few threads or strands of acid-staining material extending inward from the outer margin of the inclusion. The outer margin of the inclusion is, as a rule, more deeply stained with protoplasm or acid stains, and is seen to be composed of a thickening of the cell protoplasm at the margin of the vacuole. No capsule separate from the cell protoplasm can be made out, and the thickened protoplasm may be seen to shade off insensibly into the normal protoplasm of the cell. The staining

characteristics of cell inclusions are thus those of protoplasmic products not essentially different from the protoplasm of the cancer cell. The occurrence of chromatin in the central bodies (Greenough[2]) has been confirmed by Nösske,[3] Honda,[7] Apolant and Embden,[6] Klimenko,[4] and others. Its significance is yet under discussion. Honda and Apolant and Embden attribute the presence of chromatin in cell inclusions to the fact that a certain number of them at least are due to nuclear degenerations or to the escape of the nucleolus from the nucleus. Chromatolysis and dispersion of the chromatin has long been recognized as a form of nuclear degeneration common in carcinoma and sarcoma. Under these circumstances comparatively large droplets of chromatin are scattered through the protoplasm of the cell, and may indeed occupy vacuolar spaces in the degenerated protoplasm. The absence of a nucleus, however, and the obviously degenerated appearance of the cytoplasm, as a rule, make the identification of this phenomenon an easy one. When more than one nucleus is present in a cell, the appearances are more confusing and the correct interpretation of the inclusion is more difficult. Each such inclusion will require a special verdict. It is not improbable that appearances of this nature are responsible for such observations as that of Honda, who found inclusions in a single one of fourteen cases of sarcoma. In the majority of inclusions, however, a nuclear origin is not to be considered. The amount of chromatin is too minute, and the preservation of the nucleus and of the cytoplasm too perfect, to allow of adequate explanation on this hypothesis. The chromatin particles in the inclusions and a consideration of their significance formed a part of the writer's contribution to Report No 2[2] and need not be again detailed. A brief review of the literature upon the histology of secretion was there presented. The observations of Solger,[30] Prenant,[29] Zimmermann,[31] and other writers were briefly stated, and the relation of the centrosome to cell secretion was discussed. As a result of these observations it is apparent that the phenomenon of secretion in glandular epithelium is associated with a definite differentiated portion of the cell protoplasm,

the "ergastoplasm," with which a chromatin body, the centrosome, is intimately involved. The presence of chromatin particles in cell inclusions, therefore, far from proving their origin in nuclear changes, contributes further to the plausibility of the hypothesis that the inclusions are primarily an evidence of cell secretion.

5. *Situation in the cell:* The typical inclusion of cancer is understood to be intracellular in situation. The extracellular forms described by Plimmer,[8] Gaylord,[12] and others are extremely rare. No extracellular structures of this nature have been observed by the writer, but as has been detailed in a previous report[2] the secretion in the gland lumen of breast tumors, both cancerous and non-cancerous, in transverse section, presents occasionally appearances which resemble cell inclusions in all save the intracellular situation. It is probable that Russell's fuchsin bodies and similar structures have not been distinguished from intracellular inclusions by many of the writers who claim an extracellular occurrence of the so-called "cancer bodies."

The position of the intracellular inclusion in the cancer cell is a matter which has apparently not received especial consideration. In the 1902 report the writer presented a number of observations upon this point, and stated that in adeno-carcinoma and in non-cancerous breast tissue typical cell inclusions were found of small size and of constant position, *i.e.*, they lay between the nucleus and the free border of the cell toward the lumen of the duct. This is the position in which, in every gland cell, the products of secretion are to be found before their extrusion into the lumen of the duct. In those forms of cancer, however, where a further departure from the normal type has occurred, the gland lumen is lost and the cells appear in solid columns and masses. In such cases inclusions of larger size are found, and the relation to the polarity of the cell is lost, so that a determination of the position of the inclusion is impossible. The constancy of the lumen position of the inclusions in adeno-carcinoma and in non-malignant breast lesions has been confirmed by a large series of observations which have

been made since 1902, a number of which have been figured on Plates IX. and X. In the plates of an article by Cattle,[17] a similar distribution of the inclusions in the acinus of a breast gland at the margin of a carcinoma is conspicuous, and although the malignancy of Cattle's specimen may be disputed, the position of the inclusions, with regard to the gland lumen, is beyond discussion.

6. *Relation to mitosis and to cell proliferation:* The relation of the cell inclusions in cancer to the rapidity of growth of the tumor is of great significance in connection with the claims of the believers in the parasitic nature of these appearances. Plimmer[8] states that "in very rapidly growing (what one may call 'acute') cancers they may be found in enormous numbers." This statement the writer was forced to dispute, in 1900,[1] when the statement was made that "Plimmer's bodies" were found to be more numerous in slow growing and scirrhous forms of cancer than in the rapidly growing medullary or adeno-carcinoma forms. In 1902[2] this statement was reiterated, and the lack of association of cell inclusions and mitoses was remarked. Since that time observations by Apolant and Embden,[6] Honda,[7] Nösske,[3] and Klimenko[4] have confirmed these statements. Apolant and Embden especially make note of the interesting fact that in the small lung metastases of a breast cancer of a dog three zones of cells could be clearly differentiated — (*a*) a central, much degenerated area, without cell structure; (*b*) a middle layer of beginning degeneration with many inclusions, and (*c*) an outer layer of active growth containing many mitoses and few inclusions. The presence of many inclusions in slow growing forms of cancer is especially confirmed by Nösske's observations, and the specimens which have been available to the writer in the last two years have never failed to bear out this observation in every way.

A cancer parasite, capable of calling into activity the exaggerated reproductive power which is characteristic of cancer cells, might well be expected to occur in close relation with the cells which are undergoing mitosis and division. That the inclusions occur in inverse ratio to the rapidity of growth

and that they are practically never found in cells actually undergoing mitosis are serious deterrents to a belief in the parasitic theory or in the theory that the inclusions are due to abnormal phenomena of mitosis (Hansemann,[18] Pianese,[16] Borrel[20]). To the secretion theory, however, these observations offer marked support. Whatever the cause of cancer may be, it operates to produce an increased proliferative ability in certain epithelial cells. With the advance of proliferative power there goes a diminution of special function — the cells become "entdifferencirt" or "anaplastic" (Hansemann). The function of secretion by glandular epithelium is thus one of the characteristics which is gradually lost with the increased proliferative ability of cancerous epithelium. That this function persists for a time in perhaps an altered or abnormal state is proved by the familiar occurrence of secreted materials in the metastases of cancers of the alimentary tract, the thyroid or other glands which have a special and easily identified function. The more advanced the cancer and the more rapid the processes of cell division the less evidence of cell function do we expect. In the fact that cell inclusions are more numerous in slow growing cancers and are less frequent in advanced forms with many mitotic figures, the relation of the inclusions to the gradually departing function of cell secretion becomes again apparent.

CONCLUSIONS.

1. The typical cell inclusions of cancer are practically constant in cancer of glandular origin.

2. They are not found in epithelioma, and are almost invariably absent in sarcoma.

3. Their size, structure, and staining reactions are such as to justify the assumption that they are vacuoles in the cell protoplasm containing a material which is coagulated and shrunken by the use of tissue fixatives.

4. The occurrence of vacuoles of this nature is chiefly a phenomenon of cell secretion.

5. Similar vacuoles may be produced, however, in certain cases by phagocytosis and by degenerations of the nucleus.

6. Secretion vacuoles of the form of typical cancer cell inclusions are found in certain non-cancerous diseases of the mammary gland.

7. In such cases the secretion vacuole occupies a position between the nucleus and the lumen of the gland.

8. The inclusions in adeno-carcinoma occupy a similar position between the nucleus and the lumen of the gland.

9. In more advanced carcinoma the gland lumen is lost. The secretion vacuole cannot escape, and remains within the cancer cell to undergo further increase and ultimate degeneration.

10. Cell secretion is a function which is lost in the progressive anaplasia of cancer cells, and

11. Cell inclusions are more frequent in slow growing cancer, and are less numerous in advanced cancer with rapid cell division.

12. No reason exists for the interpretation of these appearances as of parasitic origin.

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DESCRIPTION OF PLATES.

Plate IX.

Figs. 1, 2, 3, and 4. Adeno-carcinoma of breast. Portions of tumor in which tubular formation of cells is present, showing arrangement of cells around open lumen, and situation of cell inclusions on lumen side of nucleus. Note that inclusions are of comparatively small size. Specimen No. 10.

Fig. 5. Adeno-carcinoma of breast. Mass of carcinoma cells in which two open spaces, A and B, are present. Tubular arrangement of cells around these spaces, with cell inclusions of small size on the lumen side of the nucleus. Note the larger inclusions, C, D, E, where no gland lumen is present. Note also the vacuolar character of the smaller inclusions and the absence of a central body, and compare with the inclusions of Figs. 1, 2, 3, and 4, in which a central body is present. Specimen No. 10.

Plate X.

Figs. 6 and 7. Adeno-carcinoma of the breast. Arrangement of cells around a gland lumen, and inclusions in the cell protoplasm on the lumen side of the nucleus. Specimen No. 9.

Fig. 8. Adeno-carcinoma of breast. Arrangement of cells around a gland lumen, and inclusions on lumen side of nucleus. Specimen No. 26.

FIG. 9. Cyst-adenoma of breast. Gland acinus with proliferated epithelium, and inclusions on lumen side of nucleus. Specimen No. 33.

FIG. 10. Adeno-carcinoma of breast. Arrangement of cells around a gland lumen, and vacuolar inclusions without central body in that portion of the cell toward the lumen of the duct. Specimen No. 28.

FIGS. 11 and 12. Adeno-fibroma of breast. Two gland tubules, with inclusions on lumen side of nucleus, and evidence of secretion vacuoles in the open lumen of the duct Note variation in size and in staining of central body of inclusions. Specimen No. 60.

FIG. 13. Chronic cystic mastitis. Gland acinus with proliferated epithelium. Inclusions in cell protoplasm toward the lumen of the duct. Specimen No. 38.

PLATE XI.

FIG. 14. Chronic cystic mastitis. Gland acinus with proliferated epithelium, showing earliest appearance of secretion granules in that portion of the cell protoplasm nearest to the gland lumen. Specimen No. 21.

FIGS. 15 and 16. Chronic cystic mastitis. Proliferated epithelium of small cysts, showing agglomeration of secretion granules, and beginning vacuole formation. Note the situation of these structures on the lumen side of the nucleus, and the appearance of a central body of acidophile staining reaction, with minute basophile granules in the center. Specimen No. 15.

FIG. 17. Chronic cystic mastitis. Cells from the summit of an intracystic papillary growth, showing secretion vacuoles on the side of cell toward the lumen of the cyst Note the absence of central bodies in three vacuoles, and the presence of two central bodies in the vacuole in one cell. Specimen No. 55.

FIGS. 18 to 25. From a case of adeno-carcinoma of the breast. Cancer cells with inclusions of varied sizes, showing degrees of development. Figs. 18 and 19, small vacuole without central body; Fig. 20, appearance of central body; Fig. 21, larger inclusions; Fig. 22, appearance of rayed periphery; Figs. 23 and 24, well developed, typical cell inclusions of breast cancer, showing rayed periphery and deeply-stained central body, with basophile granules: Fig. 25, large vacuole showing rayed periphery without central body, and occupying so much of the cell that the nucleus is distorted. Specimen No. 26.

(Pencil drawings by Miss Florence Byrnes of the Surgical Laboratory of the Harvard Medical School.)

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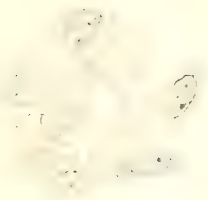

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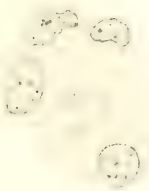

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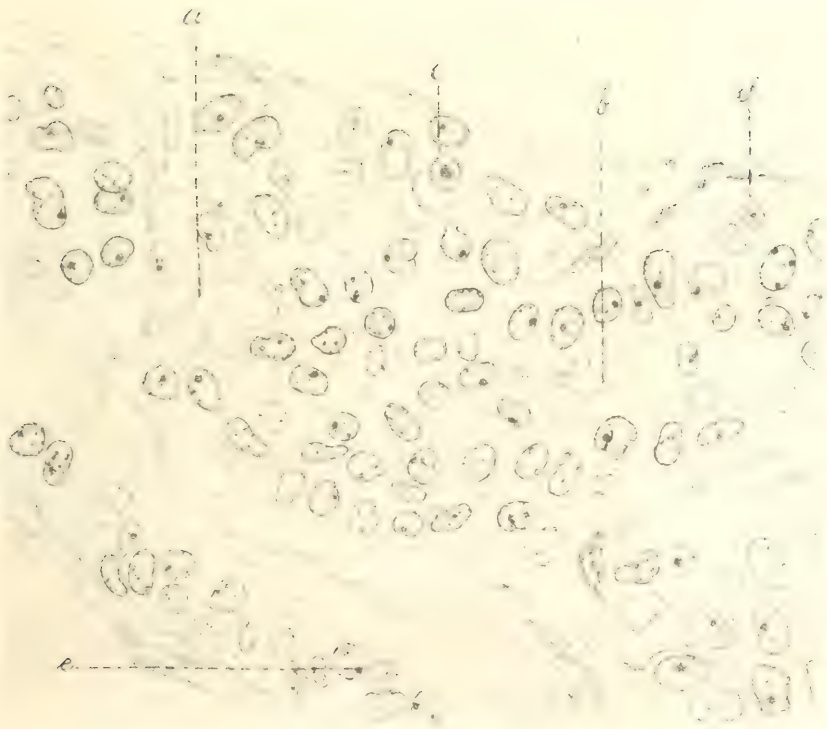

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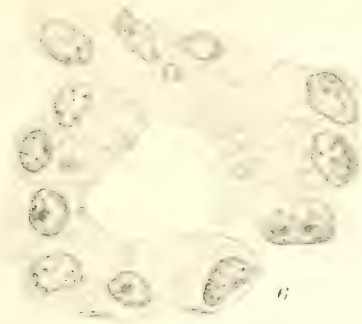

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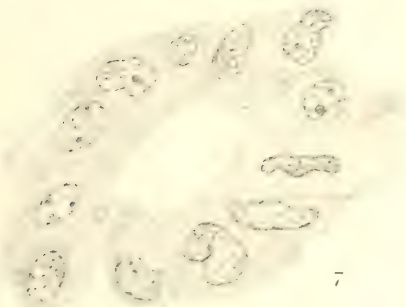

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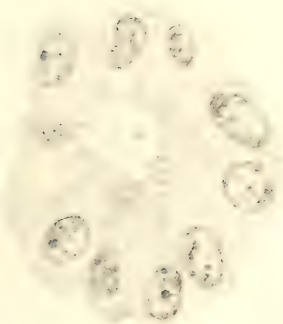

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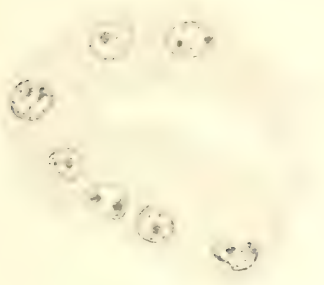
Greenough.

Cell inclusions in cancer.



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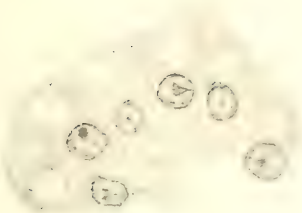

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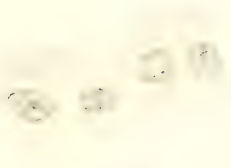

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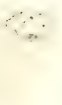

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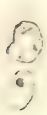

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THE EFFECTS OF THE RÖNTGEN RAY UPON CANCER.*

ROBERT H. VOSE, M.D., AND WALTER C. HOWE, M.D.

The clinical part of this investigation was undertaken and carried on almost entirely at the Massachusetts General Hospital, the material being supplied by the courtesy of the staff of the hospital. Very much of the clinical work was done by Mr. Walter Dodd, radiographer to the Massachusetts General Hospital, and his results are a part of this paper. The pathological side was done by Dr. Howe, and that part of the paper is his.

About one hundred and twenty cases in all were studied, but the results of the investigation cannot be represented by the detailing of all of the cases which have been observed, for the reason that very many of them had such a mixture of treatment that the results were not properly controlled, and many, through their inability to continue treatment, are not of use to the statistics. They have, however, added to the general impression made upon the observers, and the selected cases which are reported are chosen because they represent final accurate results and because they show some definite observation, while in none of the other cases was any observation made which is not covered in these cases.

Shortly after the discovery of the Röntgen ray it was found to affect the skin of those exposed, and its therapeutic use followed. Great claims were made for it, but at present much of the confidence at first felt has gone. Its value, however, in many skin diseases is unquestionable. The failure to effect any good in many cases of epithelial cancer and all cases of deep-lying cancer must discourage its use in any such cases.

Method of treatment.—"The key-note in the treatment of cancer by X-ray" has been said to be "sufficient X-ray to cause destruction of diseased tissue, but not sufficient to

* Received for publication Nov. 28, 1904.

destroy the surrounding healthy tissues." To arrive at this ideal performance an attempt was made at the beginning of this work to standardize the X-ray used both in quality and quantity. For this purpose the apparatus was used in as nearly similar condition as possible from day to day, although the tube could not be kept of the same quality for any considerable length of time, and a careful note was made of the varying conditions as follows: the same current was supplied, the interrupting needle was kept at the same depth in the bath, and the same coil and condensers were used. The spark gap interposed between the secondary and the tube was measured and the internal resistance of the tube measured, that is, as represented by the length of spark which could short circuit the current and shut off the tube. The patient was also exposed at a constant distance, ten inches being adopted, as showed to be a safe one in Codman's [1] analysis of X-ray burns. It was at once noted that there was marked individual variation in susceptibility, and some cases were exposed nearer to the tube, others farther from it. In general, it was noted, in accord with the practically universal experience, that the most marked effect was obtained from a tube of rather low resistance. But this was only with quite wide variations, so that tubes varying in resistance from less than an inch to two or three inches acted, as nearly as could be measured clinically, about alike on the same patient, provided that the time of exposure and the distance from the tube were the same. The estimation of this effect was made upon certain patients who felt the physiologic result almost immediately after exposure, and in whom the effect increased gradually for a while and then disappeared. That it acted in different degree in individuals was very evident, but that the index was accurate is shown by the fact that severe burns always could be avoided and that where burning was desired it was readily and certainly produced.

Clinical effect on normal skin.— Clinically, the effects of X-ray on normal skin are similar to sun-burn, and like sun-burn the result is delayed in action, the burns appearing in some reported cases as late as fourteen days after exposure.

Certain cases have been reported to become evident even later, but in these there is some doubt whether the X-ray is the sole etiologic factor. The average acute burn, meaning by this the burn resultant upon a single exposure, appears on the second or third day, and its course and duration are certainly dependent upon its severity. It appears as a blush, with some smarting or tingling and hypersensitiveness, and proceeds with gradual superficial edema, becoming blebs, which, breaking, reveal a surface of deep red, thickened, true skin underneath in a state of active inflammation. This true skin may be, in the progress of the inflammation, destroyed, leaving a superficial, necrotic ulcer which is always extremely sluggish in healing. The depth of ulceration following a single exposure is never great, though where tendons or bone are immediately subcutaneous they may be exposed. The burn may halt at any stage of the process from the faintest blush, and resolution take place. A notable feature is the delayed onset which, according to the reported cases, is not a good index of the severity of the burn, though in our experience the severer burns have appeared more promptly. When burns have not been evident in a week we have had no hesitation in increasing the number and length of exposures, stopping only when the permanent blush appeared, and we have had no unpleasant effects. The distinctive points are: first, the delayed onset; second, the remarkable sluggishness in healing, many of the ulcers taking months to heal and the more superficial burns resolving very slowy and leaving a somewhat thickened and stained surface for a considerable time.

Gilchrist,[2] in microscopic sections of the skin from X-ray dermatitis, found only chronic inflammatory changes. The horny layer was thickened, and a portion of it was detached; many brown pigment granules were found in the exfoliating portion. The mucous layer was not thickened, but it was more pigmented than normal. The vessels of the corium were dilated.

Scholtz[3] summarizes his histological findings as follows: X-rays influence especially or exclusively the cellular

elements of the skin. These are influenced primarily and undergo a slow degeneration, while the connective tissue, the elastic tissue, muscles, and cartilage are changed only in a slight degree, and suffer only secondarily as a result of the cellular degeneration and the inflammatory reaction consequent to it. The degeneration affects the epithelial cells in the highest degree, and to a less extent the cells of the glands, the vessels, the muscular tissue, and the connective tissue. The degenerative appearances are of various kinds and affect both the protoplasm and the nuclei. As soon as the degeneration of the cells has reached a certain point, an inflammatory reaction appears which manifests itself in a marked dilatation of the vessels with gathering leucocytes and marked emigration of the red corpuscles. When greater cell degeneration occurs, as a result of stronger exposure, collections of leucocytes press into a mass of degenerative cells and accomplish their further destruction. The changes in the large and small vessels are apparently of great importance as affects the further development and slow healing of the ulcerations.

Sections from the tissues of such burns studied by us show progressive changes from the surface downward, the more highly organized parts naturally showing the most marked changes or suffering most. The hair follicles and glands are destroyed. The prickle cell layer is increased. The cells of this layer show granular degeneration of protoplasm and proceed to necrosis. The blood vessels show a reticular deposit of fibrin on their inner coats. No change of nerves was noted. These histological changes are all that may be positively claimed since the sections from X-ray ulcers show a purely necrotic process — increase of elastic tissue, increase of connective tissue, and colloid replacement.

One observer has found the necrosis without marked changes in the blood vessels, and holds that the endarteritis progresses pari passu with cellular degeneration. This is opposed to the theory that the one causes the other and that the process is purely atrophic. Absence of nerve changes

removes any histological basis for the theory that the changes are due to altered nutrition through these.

Chronic burns caused by X-ray.— While all these gross and histological changes may take place as an acute burn following a single exposure, it is vastly more common to find them consequent upon multiple exposures and especially among X-ray workers who are continually exposed for short periods. To be noted in the experience of these persons are the following facts: Great variation in individual susceptibility. The difference in care and avoidance of unnecessary exposure cannot account for the immunity of some, while others suffer severely. These burns appear gradually and, the cause continuing, progress steadily, the redness of active hyperemia becoming a bronzing or yellowing (attributed by one theory to a production of nitrous acid). The nails show rugæ of malnutrition; the skin is glossy, with loss of glands and hair. The staining becomes irregular, and there is seen to be loss of pigment. Instead of exfoliating, the skin cracks and ulcers form, the granulations of which are pale and thin; tendons or bones are exposed. The sluggish response to all efforts directed toward the healing of these ulcers is remarkable. An interesting case has been reported[4] of one of these X-ray operators in whose chronic burn a typical epithelioma developed. The report goes on to say that X-ray treatment of this epithelioma caused local improvement but, glands appearing in the axilla, the area was sacrificed. This patient, according to a recent report, died about a year and a half later from extension of the disease. Another similar case has been reported, and still another has occurred to our personal knowledge. The inevitable conclusion from such cases in direct relation to this investigation will be referred to later.

Another fact to be noted is the absolute protection afforded by very slight covering. No case has been reported, nor has any occurred, so far as known, of burns except in exposed parts. This means that while faces and hands have suffered, and arms when the sleeves have been rolled up, that the

clothing, even a thin sleeve, has absolutely limited the area affected. The importance of this observation lies in the evidence thus presented that the burning effect is largely intercepted by tissue offering apparently little resistance to the X-ray.

Effect on ulcerated surfaces.— On ordinary ulcerated surfaces the effect is at first that of stimulation. This effect is clinically evident, since in most cases the surface becomes clean; the granulations become red, firm, and grow rapidly. This effect seems to take place under the eye of the observer during the exposure, although it is difficult to eliminate the effect of exposure to air and light and possibly electrical influence, for the surface at once becomes dry and glossy. The exposure thus aids by preparing the surface for healing to take place. There is no evidence to show that the X-ray has any effect on the edge of epithelium. If the exposures are continued the granulations cease to be stimulated, the epithelial edge is destroyed, and healing is retarded.

It is extremely difficult to decide at what point the beneficial influence ends, and it is for this reason the reports are varied as to the value of X-ray treatment for chronic ulcers. Another difficulty in estimating the effect of the X-ray is the varied character of ulcers treated. Since the course is quite different, it is impossible to measure with any accuracy acceleration or retardation of healing due to X-ray treatment, both in cases of rapidly healing ulcers and others of essentially chronic nature. Upon ulcerated surfaces the effect of the X-ray is similar to that on the skin — primarily a stimulation due to hyperemia; later a necrosis, acting from the surface downward and superficially limited, exactly like any other irritant, possessing a penetrating quality, but to a limited extent only; not possessing any selective action in its destructive course, for although the function of glands and hair follicles may be early lost, it is doubtful whether this is permanent unless there is other cellular destruction and the active degeneration and necrosis that sets in slightly later is general from the surface downward. Microscopically, there

is absolutely no change in ulcers to be noted that can be called peculiar to the X-ray. What effect there is has never been other than a change in degree, nor is a change in kind to be expected.

Effect on scar tissue.— Upon scar tissue the clinical effect of X-ray is peculiar. Certain cases of contracted and painful scar have softened and become much more pliable with diminution of pain. It cannot be said that this is an invariable effect, nor can any histological change be shown to explain it. Hahn and Albers-Schönberg [5] and Taylor [6] have reported the removal of scars by X-ray. Pusey [7] reports a case of keloid in vaccination scar, treated with much improvement, and says, "No scar tissue is too well developed to render success with X-ray treatment impossible."

Analgesic effect of X-ray.— Especially noted in the literature is the influence on pain. Analgesic power has been most strongly claimed for the X-ray, and it appears to be with good reason. Allen [8] has claimed that, if he can promise nothing else, he can assure his patient that the pain will be relieved. Among very many of the cases observed in this investigation, however, pain has not been a prominent symptom. Hence that power has not been largely demonstrated. A sufficient number of cases have been observed from which to draw a conclusion. Eighteen cases in which pain was marked were treated; of these, nine were temporarily or permanently benefited, in some the benefit amounting to almost total relief; five others received a similar benefit when the brush discharge from a "high frequency" machine was added, but the remaining four showed a distinct increase of pain, and at least two cases in which there was no pain at the start became painful, and two cases complained bitterly of pain coming on after exposure and lasting twenty-four to forty-eight hours. One case which showed a slight reaction after each exposure felt a tingling at the time of exposure, and this increased so as to make the patient genuinely uncomfortable, even to the extent of causing a

wakeful night following. This, however, represents the border-line case of a burn, the others not showing evidence of a burn. The cases in which burns are produced are always painful. There is no histological change in the nerves, as mentioned above, to account for this effect, but the best explanation of the analgesic effect lies in the fact that counter-irritation is often a most valuable treatment for the relief of pain.

Discussion of illustrative cases. — The following cases are described in detail in order to report all of our conclusions from this investigation. They are separated into groups which represent the varying forms of disease treated, and in the groups are contrasted individual cases in which the results were different.

Diseases of Lymph Nodes.

Case I. A B., male, 34. A non-malignant case, put in here for comparison with the following one, had tuberculous glands of the neck of considerable extent and recurrent after operation. They were hard and so close together as to make a compact mass of irregular outline on the right side, from the angle of the jaw to clavicle. He had a number of exposures lasting over a period of eight or nine weeks. The surface was brought to a condition of chronic burn, and the glands gradually grew smaller and practically disappeared. Three months later the skin was returning to its normal condition, that is, the thickening had largely disappeared; the bronzing lasted much longer, but when last seen there was no recurrence of glands. There was no histological study of gland tissue after exposure to X-ray.

Case II. L. F., male, 38. Referred for lymphosarcoma of right cervical region. Presented after operation an extensive chain of large, soft, flattened glands about right clavicle; no other glandular enlargements. The patient's general condition was good, and this tumor had suddenly appeared after strain at his work, which was farming. The process later proved itself to be Hodgkins' disease. At operation several glandular masses had been removed, leaving a deep and inoperable chain of glands. Following operation and at the beginning of X-ray treatment, this mass of glands increased greatly, becoming larger than before operation; then, under treatment, quite suddenly, it began as rapidly to disappear. The treatment in this case was carried to the point of marked erythema and no farther. It was given at intervals of two days at first, then twice weekly. At the end of three weeks there remained only a few small glands scarcely more than normal. The patient's general condition, he said, was never

better; he weighed more than at any time before in his life; slept well, had a good appetite, and was doing very hard work. At the end of ten weeks patient declared himself well, and since that time has been under observation monthly. After five months, however, glands were noted in axilla and groins; the chest was negative. After six months the cervical mass had enlarged, but promptly resolved to its former condition under Fowler's solution. At present the disease seems to be slowly progressing. It has been suggested that, in view of the hopeless prognosis of the case, an effort be made with the X-ray to destroy the local manifestation of the disease, but other experience with the X-ray does not justify such treatment.

The effect in these two cases was unquestionably due to the counter-irritation produced by the X-ray. The tuberculous glands which are so liable to break down were not degenerated by the X-ray. Hence, though enough X-ray was used to cause a chronic burn on the surface and more would have undoubtedly done serious harm, yet not enough X-ray penetrated to act destructively on the glands. In the second case the rapid increase of size was probably glandular hyperplasia, and that might well respond to treatment by counter-irritation. When these masses later increased, a similar result was obtained to a less degree by constitutional treatment.

Superficial Epidermoid Cancer.

Case III. F. G., male, 56. A case of epidermoid cancer of the nose, the duration of which was given as forty years. It had been steadily increasing twelve years, and, presumably, had only taken on epitheliomatous character clinically at date of beginning increase. It was a soft, easily bleeding ulcer, three-fourths of an inch in diameter, on the right side of the nose, and the patient complained only of its unpleasantness and frequent bleeding. The ulcer was exposed every second day in the usual way, and improvement began before any special evidence of irritation presented. Treatment was continued seven weeks, during which time the surrounding area of normal skin was burned to the extent of marked erythema and exfoliation without blistering. There was considerable itching amounting almost to pain; the growth dried and crusted. This crust was removed by anointing for several days, after which healing was complete, leaving a smooth, soft scar without hardening or contraction, and at the end of seven weeks the cure seemed complete. There has been no return.

A small piece was excised for microscopic examination, the report of which was as follows: The section consists of sharply circumscribed

masses of epithelial cells; the cells are relatively large; many epithelial pearls, in some of which the cells are loosely placed; prickle cells are easily made out. The surface of the growth is bare of epithelial covering, and shows hemorrhage and fibrin; some infiltration with leucocytes. Weigert's stain shows increase of elastic tissue. Few polynuclear leucocytes present; occasionally an eosinophile. Diagnosis: Typical epidermoid cancer.

Case IV. L. A. R., female, 71. This case presented an epidermoid cancer on the upper lid of the right eye by the inner canthus. It extended about the canthus, on to the nose, and down on lower lid slightly. It was nodular about the edges of a small ulcer, and the lid did not close perfectly. This ulcer began nine years previously, and three months before she came to the hospital she had had seventeen exposures to X-ray, with some improvement. Operative treatment would have been very deforming, and X-ray was again tried. A portion of the growth was removed, and the pathological report follows:

The section consists, for the most part, of an extensive hyperplasia of epithelial cells, collected in irregular masses; in two places the growth comes to the surface and is just covered by a thin layer of partly necrotic, squamous, epithelial cells. One side of the section shows normal epithelium, gradually thinning out as it reaches the edge of the tumor. Serial sections show the epithelial masses to be united by intermediate cellular bands. Separating the masses of cells is a moderate amount of connective tissue, rich in nuclei; blood vessels not abnormal in number, size, or condition of their walls. The cells contain small, faintly staining nuclei; there are several mitotic figures. Mitosis has been said by some observers to be absent after X-ray treatment.

The growth proved quite resistant to treatment at first, then began to ulcerate rapidly until it extended practically over the entire area of tumefaction and was rather deep. There was considerable discharge, but with frequent irrigation the ulcer was kept fairly clean and was left exposed without dressing. On account of some conjunctival irritation, several intervals of two weeks were allowed during treatment, and after nine weeks much of the whole area had healed in. The exposure was not painful, though occasionally there was felt a prickly sensation, probably not electrical, as a grounded screen did not prevent it. A month later all was healed but three small whitish nodules on the inner point of lower lid. Later these ulcerated slightly and granulated. This granular area became smaller, and presented the appearance of a slightly eroded granular lid. At present, four months since last exposure, the conjunctiva is slightly injected, and the lid does not close perfectly. The scar is smooth and soft, with no evidence of growth in or about it. The slightly eroded lower lid, or rather shortened lid, is not perfectly normal, but is, if anything, better than at the last note. A year later the patient returned with recurrent growth slightly larger than at the first visit.

Case V. A. F. D., male, 59. This man had for seven years on the left side of his neck a growing flat ulcer which had been twice curetted

and cauterized. He stated that it was the same in appearance as it had been for one year. Occasionally he had pain in it, but of little account. A portion of the ulcer was excised for histological examination, and the report follows:

The section shows a margin of healthy skin at one side; on the other the epithelium dips down into the deeper layers, in irregular thin columns. At one side is to be seen a hair follicle, with normal arrangement of cells at its base. Between the masses of epithelial cells the connective tissue shows some infiltration with lymphoid and plasma cells; apparently elastic tissue is increased. Many epithelial pearls are present, some containing leucocytes. The deeper masses of epithelial cells show necrosis and softening. A sebaceous gland shows normal arrangement of cells. Diagnosis: Epidermoid cancer.

The duration of the ulcer seemed favorable to cure by X-ray. After seven exposures the ulcer began to show the dry and glossy appearance which is typical when ulcerations react to X-ray, and soon afterwards the skin began to close in rapidly from the edges. The patient always "felt" the exposure, and the itching and burning sensation was considerable for six to twelve hours following exposure, but there was little evidence of surrounding reaction beyond slight erythema and depilation. For a while there was little change, but at the end of fourteen weeks the ulcer had completely skinned over except for three small spots which broke down at the periphery of the old ulcer. These became deep holes. The neck showed at this time a thick, slightly reddened skin depilated for a fairly broad margin about the scar of the old ulcer. The ulcer was represented by a depressed area a little larger than a half dollar, covered with tender pink skin, with, at its margin, three rather deep small ulcers with slightly thickened margins. One of these was excised and reported upon as follows:

The surface of the section at one point is covered with epithelial cells. Practically the whole section is composed of irregular masses of epithelial cells, dipping down into the deeper structures; mitoses are rare; many of the cells show necrosis and hyaline degeneration. Blood vessels show a certain amount of endarteritis. There is infiltration throughout the section with lymphoid cells and polynuclear leucocytes. Diagnosis: Epidermoid cancer.

From this time, with the hope of going deeper with the X-ray, long exposures were given, and the patient was rather severely burned, and the new skin on the original ulcer was destroyed and the size somewhat increased. The course from then on was slowly reparative to a condition nearly equal to that when the patient first presented himself. The condition remained stationary for six months more, when the patient was operated, and the growth has not returned. The excised growth was examined by Dr. W. F. Whitney, whose report follows:

A fungous growth three centimeters in diameter, surrounded by a small area of normal skin; the growth is elevated 0.5 centimeter above the

surrounding skin. Microscopically, it was found to be composed of solid masses of very large epithelial cells infiltrating the deeper tissues. Diagnosis: Epidermoid cancer.

These three cases have been put together because they seemed most hopeful and show the X-ray in the proper field, in which it has been credited with curing malignant disease. Case III. belonged to that class of chronic epidermoid cancers that does not seem very malignant and which might have responded to curetting and cautery. Case IV., which seemed at first an excellent result, recurred so soon as to have made the tedious weeks of X-ray treatment seem hardly worth while. Case V. is most instructive and demonstrates the absolute absence of any selective power in the X-ray or of any specific action on cancer cells. The X-ray does not act strongly on tissue below the surface, for clearly those cells protected slightly at the margin escaped destruction, or else being in a more active stage were more resistant and continued to grow. The other two cases bear this statement out, because both exfoliated before healing. The conclusion is that in such cases the action is purely destructive. Cells of low vitality may be destroyed on the surface, while the irritation is only stimulation to cells of higher vitality.

Recurrent Deep, Non-operative Cancers.

Case VI. Male, 59. Presented a return of cancer in the scar following an extensive operation for cancer of lip. It first appeared on right lower lip, thirteen months previous. The first operation was one year, the second five months previous, and the second recurrence appeared three weeks previous to presenting himself for X-ray treatment. There was at the first examination a firm elastic tumor to the left of the scar and behind the mental process of the jaw, extending down nearly to the hyoid bone. The tumor contained a certain amount of fluid. Following X-ray treatment, this patient had considerable pain; at the end of two weeks he had a considerable dermatitis and the treatment was suspended. Afterward he became more tolerant, but never could endure the ordinary length of exposure. The course of this case was rather rapid extension with ulceration. His complaint was "drawing sensations" upon the face and down on neck, and this he thought was somewhat relieved by the X-ray. He was anxious to continue treatment, but suffered much pain for forty-eight hours after treatment, when the exposure was very short. There was some contraction of the scar in this case which may have been slightly

relieved by the X-ray; the extension of ulceration was not rapid nor was there any heaping up of granulations, which was a marked feature of the following cases. The ulceration did, however, extend into the mouth, and was unpleasant, besides giving him great difficulty in swallowing. Constitutional failure in this case seemed in part due to starvation from this cause. Extension of ulceration seemed to give him slight relief from pain, and when last seen, seven months after the beginning of treatment, he had little pain and very extensive ulceration. A month later he died.

Case VII. J. H., male, 60. Recurrent carcinoma of lower lip. One year previously he had cancer of the lip removed. Six months later a lump appeared in the neck, and two months ago it was removed, then being the size of the tip of the finger. He presented, when coming for the X-ray at first, a tumor under the left jaw, the size of half an orange, with deep central ulceration an inch and one-half in diameter, with discharge of blood and necrotic material. It was not painful. At the end of two weeks, having had frequent and long exposures, as he was very tolerant, the appearance of the ulcer had changed in this way: it was cleaner centrally and much dryer, but still discharged yellow serum, slightly tinged with blood. At the same time the tumor enlarged peripherally, with a mounting up of the edges in a fungating "cauliflower" growth. Vigorous treatment was continued, but the general condition failed rapidly and the growth increased. A portion of the growth was removed and examined histologically.

The section at one point shows loss of epithelium, beneath which point there are irregular thin columns of epithelial cells penetrating the tissue beneath. Between these masses are plasma and lymphoid cells and an occasional eosinophile. These masses of epithelial cells extend laterally beneath healthy skin; mitoses are numerous; there is increase of elastic tissue; marked endarteritis is present. Diagnosis: Epidermoid cancer.

The area about the ulcer was depilated and erythematous, but not severely burned. Omission of treatment for four days brought on pain. This growth was evidently stimulated by the treatment, for the heaping up of grayish, foul granulations, discharging a yellow serum, came to measure nearly seven inches across. The patient died four months after beginning treatment.

Case VIII. G. O. A., male, 39. Recurrence of carcinoma of lip. This patient had had a small ulcer on his lip for ten months, and for three months a "lump" under the jaw. It was operated on at the hospital, and being too extensive to admit a complete operation, was at once referred for X-ray treatment. The wound of the operation had healed, but the scar was adherent and thickened. Upon X-ray treatment the scar softened somewhat, but as he was quite sensitive to the X-ray the exposures had to be at rather long intervals. At the end of one month a spot in the scar was softened so as to appear about to break down, and patient disappeared for three weeks, when he returned with a fungating recurrence. At the surgeon's request he was given frequent long exposures, and in a week the tumor began to ulcerate. He had no pain, and

said he felt better. X-ray treatment was continued until he was severely burned, the ulceration increased, and the disease itself seemed to be rapidly growing. Pain became a prominent symptom, was not relieved by X-ray, and a month later patient thought that a bloody discharge was increased by the exposures and wished to discontinue treatment. From this time on pain was only relieved by morphia, and patient died seven months after beginning treatment.

Case IX. D. C., male, 85. Carcinoma of lip. This patient had had a growth removed from his lip twenty years ago. Six months ago an ulcer returned. He had a flattened, ulcerated area on lower lip from the corner of mouth to the middle of the lip. It seemed rather deep, had a tendency to form crusts, and extended slightly into the mouth at the corner. After two weeks of X-ray treatment it appeared somewhat necrotic on the surface and increasing in size. During the next week there appeared slight evidence of burn, but apparent improvement of the ulcer, and after an interval of three days the change for the better was surprising. A week later, however, with improvement of the lip, the corner of the mouth thickened up and the growth evidently increased at this point. Improvement stopped here, though for two months there was little change. The last note was of a rapid increase, and the patient was lost sight of.

A small specimen removed while treatment was progressing shows microscopically no evidence of carcinoma except at the edge, where beneath the epidermis there are several small islands of epithelial cells. Mitotic figures appear among them. The surface of the ulcerated area shows more or less fibrin, enclosing leucocytes; below this is granulation tissue, containing many leucocytes, and still deeper are to be seen several epithelial pearls surrounded by young connective tissue. The elastic tissue is increased. Diagnosis: Epidermoid cancer.

Case X. J. W. M , male, 36. Epithelioma of lip. This was of six months' duration, appearing first as a blister on the lip. He presented a flattened, dry ulcer extending over the left half, with a crust extending over two-thirds of the whole lower lip. No pain. A piece was excised for histological examination, and the report follows:

The section shows only the edge of the growth; the surface is covered with epidermis; the corium is densely fibrous; there are a few striated muscle fibers at the lower edge of the specimen. The corium at one end contains small masses of epithelial cells. Prickle cells can occasionally be made out. The connective tissue shows some infiltration with lymphoid and plasma cells. At points where the muscle tissue is invaded the fibers are undergoing simple atrophy. The elastic tissue shows increase. Mitoses are frequent; several in one field. The epithelial cells show degeneration in places, and are hyaline in appearance. Epithelial pearls are numerous. Diagnosis: Epidermoid cancer.

There was slight evidence of burning at the end of two weeks and some clinical improvement. From this time on the change was gradual. The lesion gradually healed toward the center of the original area till it measured one-half inch in diameter, and appeared as a dry, verrucous

excrescence which very slowly increased and seemed resistant to the treatment. It seemed that a much less extensive operation than was at first indicated would be successful, and at the end of four months he was seen by Dr. Warren, who advised this; but the patient had heard of a "plaster" that could cure cancer and decided to try it. This patient's lip, according to his own history, exfoliated two months later, and is now apparently well.

This case has been put with the four preceding it because it appears that rapid growth is not necessarily a sign of malignancy, but in certain cases may be only a surface growth amenable to X-ray treatment. Cases VII. and VIII. are put together to show that it is not a mere matter of amount of exposure that is accountable for failure, for Case VII. had a justifiable amount and not too much, with only stimulative action. Case VIII. had an extreme amount and also was stimulated. There does not seem to be a happy medium. Case X. showed exfoliation and healing of a part of the ulcer, while part heaped up, as sometimes happens in X-ray treatment of less serious skin lesions. The results shown in these five cases may be explained by the superficial irritant effect already admitted to belong to the X-ray, and nothing in these cases points to any other action.

Deep Cancer, Primary.

Case XI. S. C. H., male, 48. Carcinoma of jaw, which began as a small ulcer inside the mouth six months before and had been operated on three months before. He presented a small sinus in a scar on the right cheek leading into the upper maxilla, where bare bone could be felt. A sinus also opened into the mouth. A curetting from the sinus was examined histologically.

Part of the growth is distinctly adenomatous, showing small alveoli lined with layers of epithelial cells and well separated by bands of connective tissue. The greater part, however, is not adenomatous, but consists of small alveoli and much stroma. The cells are relatively small; mitotic figures are abundant. Infiltration with lymphocytes in several areas; also areas of hemorrhage. Diagnosis: Carcinoma.

The patient had one month of X-ray treatment, and during that time the following notes were made: Pain was present at the beginning, and continued more severe if anything, under treatment, for two weeks; on the sixteenth day the sinus on the cheek closed and there was a marked diminution of pain, replaced by a "drawing sensation" in the neck.

A week later there was a beginning erythema of the surface exposed to the X-ray, but at the end of the month the sinus had reopened. There was much increase of pain, and he was readmitted to the hospital, where a second operation revealed a very extensive growth, microscopically of similar appearance to that of the original disease.

Case XII. S. McL., female, 73. Carcinoma of right breast. A large section had been removed and the patient presented a linear scar over an irregular tumor in the upper segment of right breast — hard, non-sensitive, not adherent. She had at first some pain and drawing sensations after exposure of X-ray, but this soon ceased. At the end of two weeks a slight burn appeared and blistered about the healed incision. Later, this burn became so severe as to necessitate suspension of treatment, and the patient also complained of dragging pain. When treatment was resumed it was continued with minor interruptions for six months. The skin became more tolerant, and she had increasing doses of X-ray, but not very long or frequent exposures. The changes from time to time were inconsiderable, but toward the last there was noted a slight increase in size and adherence of the overlying skin till it seemed that sooner or later it must ulcerate, and the patient was persuaded to accept operation. Microscopically, the section consists of

I. Dense fibrous tissue containing in its meshes masses of closely packed, large epithelial cells. These masses vary greatly in shape and size. Their borders are separated from the fibrous stroma by a narrow clear space. The nuclei are large, vesicular, round or oval, and lie in close contact. An occasional mitotic figure is seen; the cell outlines are indistinct. There is extensive degeneration in places, and surrounding some of these are many compound granule cells, with reticular protoplasm. The fibrous stroma contains many plasma cells and lymphoid cells, the latter chiefly in groups.

II. Section of skin of axilla and underlying tissues, showing a large area of dense fibrous tissue in the deeper parts containing many foci of lymphoid cells and few plasma cells. At one point is a collection of yellow refractive pigment granules, both intra and extracellular, due to old hemorrhage. There are two ducts lined with epithelial cells, identical in appearance with the cells found in the breast; in places these cell masses project into the lumen of the duct. The duct contains large desquamated epithelial cells. Diagnosis: Carcinoma.

These cases are detailed to show that while the X-ray is acting on the surface, as in its effect in closing the external sinus in Case XI., or as a powerful burning agent on the skin in Case XII., its deep effect was so slight as to be unrecognizable in either case.

Abdominal Cancers.

Case XIII. D. C., female, 46. This patient had a total hysterectomy for cancer of the uterus one year previous to being referred for X-ray treatment. She did not know when the tumor had reappeared, but presented hard, fixed, lobular tumors filling the pelvis and extending on the left upward to the level of umbilicus. Pain and tenderness and all the accompanying discomforts of such growths were present, but patient showed a surprisingly good general condition.

The X-ray in this case never had any local effect on the tumor masses. It was carried to very slight erythema of the skin of the abdomen. However, the patient felt for a time much improved, had less pain and discomfort, but later she had exacerbations without X-ray treatment, and the benefit must be attributed to slight counter-irritant effect and mental improvement. She died three months later.

Case XIV. Recurrence in the unhealed sinus of cancer of colon. Operated on in August, he presented in the following January a very necrotic mass of granulations in the scar of a laparotomy wound. The X-ray had no apparent effect on this mass, though faithfully used for one month, when the patient went home and was lost sight of. He did, however, think that his general condition improved. But it would hardly be justifiable to attribute that to the treatment.

These two cases are at variance with the reported cases of cancer of the cervix and intestine greatly benefited by the X-ray.

Histological effect of X-ray on cancers. — Various histological changes in cancer produced by the Röntgen ray have been described by different observers. All observers agree that there is a degeneration of the cellular elements. Walker[9] studied sections of a rodent ulcer healing under treatment, and described the new growth as undergoing fibro-myxomatous degeneration. McCaw[10] found a colloid change in an epithelioma of the uvula. Beck[11] described a colloid degeneration in an adeno-carcinoma. Sequeira[12] observed a destruction "by lysis" of epithelial cells, involving the nucleus and the protoplasm; in some cells there was a definite fatty degeneration.

The earliest change in cutaneous cancer is in the outermost of the groups of epithelial cells, and seems to be a hyaline degeneration. Ellis[13] summarizes his histological findings as follows: He finds necrosis of cells and trabeculæ

of varying degree; increase of elastic tissue (in three cases) both before and after exposure; fewer areas of lymphocytic infiltration in one case after exposure; about equal numbers in other cases; a tendency to occlusion of vessels by deposits on inner surfaces; practically entire absence of infiltration by polymorphonuclear leucocytes. Mayo [14] found a large number of leucocytes; he observed that the vessels were dilated. Stewart [15] observed in four cases marked fatty degeneration; fat was present "in a large percentage of the epithelial cells, and this infiltration seems to increase with the number of exposures to the rays." He also noted hyaline degeneration in both pearls and single cells; that karyokinesis entirely disappeared under treatment; that there was present a considerable amount of elastic tissue, and that soon after treatment was begun there appeared marked evidence of the formation of new blood vessels. Newcomet, [16] after sixteen treatments of epithelioma of the face which was healing, found microscopically no change except some slight degeneration of the cells. In another case of epithelioma of the face, of thirty years' duration, after three months' treatment with X-ray, no change was observed histologically, although the ulceration was closing in.

CONCLUSIONS.

From this investigation it seems that cutaneous cancer treated by X-ray undergoes a degeneration, not peculiar to this form of treatment, or distinguishable histologically from degeneration from other causes. The vascular changes are limited to an endarteritis; new formation of blood vessels occurs if healing takes place, as in the process of repair elsewhere; there is an increase of elastic tissue. Mitotic forms are less abundant after treatment.

Clinically, taken as a whole, the clinical cases show:

(1.) That the only cure of cancer by the X-ray is by destruction and exfoliation. This at once limits its value to superficial cases.

(2.) That this destructive process is a slow one and acts very superficially. Since it is well known that many

essentially chronic superficial epidermoid cancers may be removed permanently by the slightest surgical procedure, that course seems preferable to the somewhat tedious treatment by X-ray, and as they both may fail, an extensive surgical operation, if necessary, may be undertaken more promptly in the former case.

(3.) That being non-selective in its action, the X-ray cannot be used strongly enough to effect destruction of anything but the shallowest tumors without serious injury to the overlying and surrounding tissues, or, in other words, producing such a burn as experience shows in all probability never would heal.

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IMPLANTATION OF TISSUE AND ITS RELATION TO CANCER.[1]

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Below are presented the results of a series of implantations of tissue in sixty-two animals. For implantation both adult and fetal tissues were used. The implanted tissues were chiefly epithelial, although some mesenchymal tissues were used. The experiments were undertaken to see if epithelial or mesenchymal tissues, set free from their normal environment could acquire a power of unlimited growth and give rise to the formation of metastases. In some cases the implanted tissues proliferated, maintained their vitality, and produced nodules analogous to dermoid cysts or teratomata, and some of the nodules were of large size. In no case was any nodule produced with unlimited growth or the formation of local or glandular metastases.

This line of experimentation was undertaken because, from a previous study of the inclusions in cancer cells, from experiments with the so-called "cancer yeasts," from a study of various lesions claimed to be due to protozoic infection, and from a study of the literature on the infectiousness of cancer, it was believed that there was less evidence that cancer was due to a parasite than that it was due to some inherent character of the epithelial cells. A critical review of the reasons which led to this belief follows.

The investigators of the subject of the origin of cancers and other malignant tumors may be divided in two classes. One class believes that cancer is due to a living parasite, the other that the epithelial proliferation in cancer is due to some biological peculiarity of the cells themselves. By those who believe in the parasitic nature of cancer certain general

[1] Received for publication Nov. 15, 1904.

reasons are advanced to support the general assumption. The men who accept the parasitic theory of origin may be divided into two classes, some believing that the parasite is a blastomyces, others that it is a protozoon. By those who believe that the development of cancer is due to biological causes, various hypotheses, supported by various known clinical and experimental facts, have been advanced. The hypotheses which have found widest acceptance are those of Thiersch, Cohnheim, and Ribbert, while with these three should be mentioned the theory recently advanced by Bashford and Murray. The evidence for and against all of these ideas will be taken up in the order in which they have been mentioned.

I. GENERAL ARGUMENT FOR THE PARASITIC NATURE OF CANCER.

1. *Apparent Relative Increase in Frequency of Cancer.*

If a relative increase in the number of cases of cancer could be demonstrated, it would tend to show that the disease was infectious. It is claimed that there is such a relative increase, and that the increase is general in all countries; that there is also a local increase in certain regions, and that certain houses are especially associated with the occurrence of cases of cancer. The statements are based upon a study of statistics. The inherent weakness of statistics is too evident to require comment. It is noteworthy that statistics from men who favor the parasitic theory are invariably in favor of their contention. The relative value of statistics in support of such a theory must in the nature of things be of small, and, if not verified by other facts, of almost no value. Without going into a detailed comparison of the various published statistics, it may be said that the result of statistical evidence is about as follows:

In earlier days the term "cancer" included many lesions, the cause of which is now known. This removes such lesions at present from the class of cancers. This fact *per se* should

lessen the apparent number of cancers. On the other hand, from improved clinical diagnosis, and from the frequent occurrence of pathological and histological examination, many deaths, formerly obscure, are now classified as due to cancer. Moreover, an improvement in the requirements regarding the registration of the causes of death by many countries has led to an increase in the number of cases of cancer reported. It has been shown [1] that the relative number of cancers is greater in cities than in rural districts, probably because of better registration and the greater likelihood that the cases will be seen by physicians. There also has been an increase in longevity on account of the lessened death rate from acute infectious diseases. Since cancer notably is a disease of later life, this would explain some of the apparent increase in frequency. Finally, as Newsholm [2] remarks, the statistics as to the cause of death represent the clinical opinion of medical men and not the facts, *e.g.*, Bollinger [3] shows that of seven hundred and eleven cases of cancer seen at autopsy at the Friedrichshain Hospital, in twenty-two per cent no diagnosis of cancer had been made during life. Newsholm believes that the increase in cancer is only an apparent one, due to improved diagnosis and better registration, but that absolute proof on either side is impossible because of the imperfect data available. He believes that there has been no real increase during the last forty years.

Statistical evidence of local frequency.— It has been claimed from a study of statistics that certain local causes favor the occurrence of cancer, and this fact has been considered to show that cancer is infectious. Wolff,[4] *e.g.*, showed that the distribution did not depend upon geographical conformation, rain fall, or elevation, but that cancer was apparently more frequent in great river valleys and in wooded districts. Bashford

[1] Bollinger. Die Haufigkeit des Karzinoms in München. Münch. Med. Wochenschr., 1903, No. 38.

[2] Newsholm. The statistics of cancer. The Practitioner, 1899, p. 371.

[3] Bollinger. Loc. cit.

[4] Wolff. A comparative study of cancer mortality. Brit. Med. Jour., 1903, p. 929.

and Murray[1] have shown, however, that cancer occurs in all of the domestic animals and in various wild animals, including mammals, birds, reptiles, and fishes. This certainly suggests that, on account of the varying conditions of life and environment under which these different animals live, some general cause must be found, and that in all probability no local cause can be operative. Bashford and Murray also show that cancer occurs in all countries, although it is apparently less frequent in the less civilized ones, and believe that this is due to less careful observation.

It also has been claimed by many that cancer occurs more frequently in certain local districts, and,[2, 3, 4] *et al.*, more especially, in certain houses. On this subject again there is a dispute as to facts. The wide zoölogical distribution of the disease as just mentioned would make such a local increase theoretically unlikely, and Newsholm says that "observations of considerable magnitude, making all necessary corrections for age distribution of the population, would be required to give a *prima facie* case to establish a high degree of probability for the contention that there are special 'cancer houses,' and such observations as have hitherto been published do not carry conviction to my mind."

On the whole, it may properly be said that statistics *per se* must be considered a weak support for the theory of the infectiousness of cancer; that there is a dispute as to the accuracy of the facts and to the conclusions which may be drawn from them; that there is no positive evidence that there has been a relative increase in the number of cases of cancer not explicable on other grounds than infectiousness, and that there is very good evidence that local causes are not active in the causation of the disease.

[1] Bashford and Murray. The zoölogical distribution of malignant new growths. Sci. Rep. Cancer Research Fund, No. 1, London, 1904.

[2] Lyons. A statistical study of a rural cancer district in the State of New York. 4th Ann. Rep. Cancer Lab., N. Y. State Board of Health, p. 97.

[3] D'Arcy Power. A further contribution to the distribution of cancer. Practitioner, May, 1903.

[4] Behla. Ueber die Krebserkrankungen in Luckaü. Zeitschr. f. Med., 1901.

2. *Formation of Metastases by Cancers and other Malignant Tumors.*

In many of the infectious diseases due to a parasite, *e.g.*, to bacteria, by distribution of the parasite through the blood or lymph vessels, secondary foci are produced in parts of the body at a distance from the original point of infection. It also is well known that a primary nodule of cancer or sarcoma may be followed by secondary nodules in various parts of the body through extension. This occurrence of secondary nodules in cancer has been claimed to be analogous to the metastases in known parasitic diseases, and has been claimed to indicate the infectiousness and parasitic origin of cancer.

In fact the character of the metastases in cancer is probably the most convincing theoretical argument against the parasitic nature of the disease. Identity of method of extension of two diseases is no proof of identity of origin. It has been shown,[1] *e.g.*, that normal tissue introduced into the circulation may be carried to various parts of the body at a distance from the original point of inoculation, may lodge and grow. In bacterial diseases the primary area of reaction is due to reaction of the fixed tissues to the irritation produced by the bacteria. The secondary areas, or the metastases, are produced by the reaction of the fixed tissues of that part of the body to which the bacteria or irritant is carried, but do not represent a transfer of the cells of the primary nodule. In cancer the facts are very different. The secondary nodules always represent a transfer and proliferation of the cells from the primary nodule, and are not produced by the reaction of the fixed cells of the part, *e.g.*, if, with an epidermoid cancer of the lip, metastases are formed in the liver, the metastases are always identical histologically with the primary nodule. If the disease were due to a parasite which had the power of producing an unlimited proliferation of epithelial cells, the secondary nodule in the liver should be composed of cells resembling the liver epithelium Such never is the case, the

[1] Cohnheim and Maas. Zur Theorie der Geschwulstmetastasen. Virch. Arch., lxx.

histological type of the original nodule is invariably reproduced. To explain this reproduction of the original type on a parasitic basis it would be necessary, as Ribbert has shown, to assume that the parasite which produces a given type of epithelial proliferation of cancer must be specific for that type of epithelium only, that it must always live within those cells, and must divide at the same rate as the type of cell within which it lives. Since cancers occur resembling all types of human epithelium, this would require just as many types of specific cancer parasites as there are types of human epithelium. Moreover, since it has been shown that cancer is widely distributed in the animal kingdom, and since, as will be shown later, there is no proof that cancer of one species of animal can be transferred to an animal of another species, just as many specific parasites would be required as there are types of epithelium in all of the various species of animals in which cancer occurs. A priori such a series of parasites seems improbable at least. Moreover, this assumption does not agree with the statements regarding the occurrence in the tissues of various alleged cancer parasites. Roncali, *e.g.*, claimed to produce an adenocarcinoma, an epidermoid cancer, and a sarcoma with one and the same parasites. Gaylord [1] claims to have produced epithelial proliferation in the lung by injection of ascitic fluid from abdominal cancer, and to have had cancer of the liver follow inoculation with ascitic fluid from an abdominal cancer. He also claimed to find the "parasite" in the circulation, in effusions, and in the tissues. von Leyden [2] claims to have found the parasite outside the cancer cells, and in a previous report by the Cancer Committee of the Harvard Medical School the bodies claimed to be parasites have been seen outside cancer cells.

In this connection it should be borne in mind that Locke [3] showed that the growth of cancer is by direct extension of

[1] Gaylord. On the presence of a characteristic organism in cancer. Third Ann. Rep. N. Y. State Path. Lab., Buffalo, 1901.

[2] von Leyden. Zur Ætiologie des Carcinoms. Zeitschr. f. Klin. Med., xliii, p. 1, 1901.

[3] Locke. The reconstruction in wax of a nodule of cancer. First Ann. Rep. Cancer Committee, Harv. Med. Sch., Boston, 1900, p. 39; Journ. Bost. Soc. Med. Sciences, v.

the cancer cells from one focus, and not by extension from various foci. Petersen,[1] after similar study, came to the same conclusion. This fact would argue against a proliferation of epithelial cells produced by a parasite disseminated outside of the cells, attacking several scattered areas at approximately the same time.

3. *Occurrence of Cachexia in Cancer.*

In patients suffering with cancer there occurs a peculiar cachexia, which often appears to be out of all proportion to the size of the tumor. Since in some diseases known to be due to a parasite a similar cachexia exists, it has been assumed that the cachexia in cancer also indicated the presence of a parasite. Clinically, however, the amount of cancer cachexia is very variable; it may be absent or marked with a small cancer, or slight with a large one. There is much clinical evidence to show that the amount of cachexia is dependent upon the site of the cancer; it is greatest in cancers which ulcerate early or are so situated as to give a chance for secondary bacterial infection, rather than due to the primary effect of an alleged parasite.

4. *Possibility of Direct Transfer of Cancer from Person to Person.*

It is claimed that it is possible to transfer cancer from a person suffering with the disease to a well person by direct contact or inoculation. Behla[2] reports seventeen cases, chiefly of his own observation, of what he considers transfer of this sort, and believes that these cases argue the infectiousness of cancer. An analysis of his cases shows that in but a small number was the affected epithelium or the cancer in the two individuals of the same type. In most cases the type of cancer was different in the two individuals, *e.g.*, cancer of the stomach in one, and epidermoid cancer of the lip in the other, etc. Bearing in mind the nature of cancer

[1] Petersen. Ueber Aufbau, Wachstum, und Histogenese der Hautcarcinome. Beiträge z. clin. Chir., 1902, xxxii, p. 3.

[2] Behla. Über "Cancer à deux." Dtsch. Med. Wochensch., 1901, xxvi.

metastases, already mentioned, the infection in these cases does not seem proven. Boas[1] reports five cases in which both husband and wife had cancer of the stomach Elsler[2] reports a case of cancer "à trois" — the first individual having a cancer of the rectum, the second cancer of the lip, and the third cancer of the rectum. Guermonprez[3] reports the case of a surgeon who examined a woman with cancer of the uterus while he had an acne pustule on the finger. Afterwards the surgeon had a cancer of the finger. Guermonprez himself operated on a cancer of the face and injured his finger. A nodule afterwards appeared on the finger which resisted the cautery for nine months.

Budd[4] reports a nurse who washed the linen of a woman with cancer of the uterus, who afterwards had cancer (sic!) of the axilla. The cases can be indefinitely multiplied, but the character of the evidence can be judged from the examples given. These cases can be divided into four groups, where people living together in close association are affected by (1) cancer of the same type where direct contact is possible, *e.g.*, cancer of uterus in wife, with epidermoid cancer of penis in the husband; (2) cancer of the same type or organ where direct contact is not possible, *e.g.*, husband and wife each having cancer of the stomach; (3) cancer of different types where contact is possible, *e.g.*, cancer of the breast in one and cancer of hand in the other, and (4) cancer of different types where no contact is possible, *e.g.*, cancer of stomach in one and cancer of liver in another. The first class, which is the most suggestive of all, is extremely rare, and when one considers the frequency with which, *e.g.*, both uterus and penis are affected by epidermoid cancer, it is obvious that such cases are quite as readily explained by coincidence as by infection. Moreover, considering the latency of many cancers of the uterus, and the fact that

[1] Boas. Statist-Klinisch. Studien an 200 Fällen von Krebs des Magen-Darmkanal. Verhandl. des xviii; Congr. f. inn. Med. zu Wiesbaden, 1900, p. 374.

[2] Elser, quoted by Behla. Loc. cit.

[3] Guermonprez. De la contagion professionnelle du cancer. Bull. Méd., 1896, p. 251.

[4] Budd. Is cancer contagious? Lancet, 1887, ii, p. 1091.

unsuspected contact cannot be rare, the evidence of infection is slight. In the second class coincidence again seems as justifiable an explanation as infection, especially in view of the rarity of the cases. As evidence in so complicated a subject as cancer such cases at best seem only suggestive, and, as proof, are too trivial in view of other evidence. In the third and fourth classes the improbability of infection in view of what has been said in regard to cancer metastases is very great. On the whole, it can be said that evidence of the transfer of cancer by contact is slight, and proof is absolutely lacking. Foulerton,[1] in a discussion of this subject, says that "there is no evidence worth considering that there is such a thing as direct personal infection."

5. *Self-inoculation by Cancer.*

The transfer of cancer from one part of the same individual to another by transplantation has been demonstrated. Cornil[2] reports two cases which were told to him by the surgeon who did the transplanting. In one case a woman had a fibro-sarcoma of the breast, which was removed by operation. A bit of the tumor was buried in the second breast, and a tumor developed at the point of inoculation with the same histological structure as that of the original tumor, with many mitoses present. In the second case a woman had a tubular cancer of one breast, which was removed by operation, while a bit was implanted in the sound breast. After some months a tumor with structure similar to that of the original tumor developed at the point of inoculation. Cornil expressed his great disapproval of the experiments. Hahn[3] transplanted a bit of cancerous skin to another part of the same individual and witnessed a cancer of the original type develop at the point of inoculation. Moreover, similar implantations have occurred

[1] Foulerton. The causation of carcinoma and sarcoma. Practitioner, 1902, July, August.

[2] Cornil. Sur les greffes et inoculations de cancer. Bull. de l'Acad. de Méd., 1891, No. xxv.

[3] Hahn. Ueber Transplantation carcinomatöser Haut. Berlin. Klin. Wochenschr., 1888, xxv, p. 413.

accidentally during operations upon cancerous tumor. The recurrence of malignant tumors in the scar of the operation at some distance from the original location is well known, and frequently has been reported. Richardson[1] reports an infection of the pectoral muscle with cancer by the use of a trocar to remove a bit of tissue from a breast tumor for histological examination. Implantation of cancer by contact in the same individual also occurs with reproduction of a tumor of the original type, *e.g.*, epidermoid cancer of the lower lip may cause a cancer of the upper lip if the two are in contact. The same thing is true of the female genitals, etc. Ebert[2] reports twenty-two such cases, but believes that they all can be explained by accidental implantation, and that they do not represent an infection of adjacent epithelium by a parasite, and therefore cannot be used as an argument for the infectiousness of cancer. In all of these cases it is to be remembered that the original type of epithelium always is reproduced.

6. *Direct Inoculation of Human Cancer into Animals.*

There are many alleged cases of the direct transfer of human cancer to animals, and this fact has been used as evidence of the contagiousness of cancer. Langenbeck,[3] in 1840, injected the juice of a medullary cancer of the humerus (sic!) mixed with defibrinated blood into the femoral vein of a dog. The animal died in two months, and showed nodules in the lung which histologically were "carcinomatous tubercles." There are no drawings, and the description of the histology makes it probable that the nodules were probably areas of granulation tissues and giant cells about cancer cells acting as foreign bodies. There is no evidence of proliferation of the transplanted cancer cells or of true tumor growth. Follin[4] injected into the jugular vein

[1] Richardson. Bost. Med. and Surg Jour., cxxxix, p. 414, 1898.

[2] Ebert. Zur Casuistik des Infectiostät des Carcinoms. Inaug. Dissert., Berlin, 1895.

[3] Langenbeck. Schmidt's Jahrb., xxv, 1840.

[4] Follin. In Lebert's Traité pratique des malades cancéreuses. Paris, 1851, p. 136.

of a dog an emulsion from a cancer of the breast. The animal died in fifteen days, and showed nodules on the walls of the heart and in the liver in which "cancer cells" could be seen. Here again there is no evidence of growth of the cancer cells, which probably acted as foreign body emboli. Lanz[1] inoculated guinea-pigs with an emulsion of a melanotic sarcoma, and in one animal found many pigmented cells, but did not produce a tumor of the original histological type. Francotte and de Richter[2] inoculated ten white rats subcutaneously in the breast with cancer juice. Each animal was inoculated with juice from seven different tumors of varying histological structure, epidermoid, scirrhus and encephaloid, at different intervals, extending over a period of six months. No result was obtained until three months after the last injection. Then at varying intervals a nodule of new tissue appeared in each of the animals near the point of inoculation, which in most cases led to necrosis and spontaneous amputation of the adjacent limb. After this amputation the animals usually recovered their general health. The observers studied the tissues in two of the animals. In one case they found an epidermoid cancer, with similar metastases in the adjacent lymph nodes. In another case they believed they saw an alveolar cancer of the breast, without metastases in lymph nodes. The paper was presented as a preliminary report, and the writers were to study the nodules in the remaining animals. No report has yet appeared, so that it is fair to presume that in the other cases no cancer was found. Transplantations to other animals were attempted. There is no proof that the cancers came from the inoculated cancer juice. Mayet[3] inoculated rats with a glycerine emulsion of human cancer tissue, and in one animal found a cancer nodule in the kidney. There is no evidence that this nodule was the result of the inoculation. Boinet[4] inoculated sixty animals with

[1] Lanz. Kocher's Festschrift. Wiesbaden, 1891.

[2] Francotte and de Richter. Récherches experimentelles sur le cancer. Bull. de l'Acad. Royale de Med. de Belgique, xliv, p. 999, 1892.

[3] Mayet. Sem. Méd., 1893, p. 283.

[4] Boinet. Transmission aux animaux du cancer de l'homme. Sem. Méd., 1894, p. 213.

human cancer tissue. All subcutaneous inoculations with masses of tissue failed. In one case a rat inoculated in the pleura with the juice of a lymphosarcoma showed a nodule of cancer (sic!). He also inoculated a rat peritoneally with tissue from a cancer of the penis, and after two months found two cancer masses the size of a cherry below the diaphragm, attacking the vertebræ, compressing the cord, and causing paralysis. Here again is no evidence that the tumor was derived from the implanted tissue Bosc and Vedel[1] inoculated rabbits with human cancer tissue, and got at the point of inoculation "new formations," adherent to the skin with enlarged adjacent lymph nodes. Where the nodule was adherent to the skin there was a proliferation of the Malpighian layer, and many "cancer bodies" were seen. By similar peritoneal inoculations they got nodules in the liver and spleen composed of "connective tissue and large nucleated cells." They inoculated guinea-pigs with the tissue of a melanotic sarcoma subcutaneously and intraperitoneally, and obtained nodules "with the structure of melanotic sarcoma." They also inoculated dogs peritoneally. In one case they put tissue from a cancer of the lip into the peritoneal cavity, and after two months found a nodule like that of the lip in process of evolution. In another case, after inoculation with tissue from a cancer of the breast, they found a large nodule the size of an orange; a bit of this nodule in the second dog was followed by the formation of a large nodule, and a piece from this second nodule in still a third dog showed disseminated nodules on the peritoneum. In this case there is no histological evidence of the cancerous nature of the nodules. They also inoculated a dog in the peritoneal cavity with a large piece of encephaloid cancer, and in two and one-half months found a nodule of "alveolar structure" with many cancer bodies. In none of Bosc and Vedel's cases is there any histological evidence of the correctness of their claims. Reale[2] transplanted a bit of skin sarcoma into a rabbit's subcutaneous

[1] Bosc and Vedel. Sem. Méd., 1898, p. 166; Presse Méd., 1898, p. 213.

[2] Reale. Tentativo d'inoculazione sperimentale del sarcoma sutaneo. Naples, 1902.

tissue, and after two years a nodule was found which histologically was a lymph-angio-sarcoma, *i.e.*, not of the original type. Dagonet[1] inoculated the peritoneal cavity of a rat with tissue from a pavement-celled cancer of the penis. The animal died in eighteen months with gradual emaciation. Nodules were found in the omentum, spleen, and liver, but the peritoneum was normal. The nodules histologically were pavement-celled cancer. In this case there is no proof that the nodules came from the transplanted tissue. Many men have reported absolute failures in attempts to transplant human cancers to animals. I have made between twenty and thirty such attempts, using rabbits and guinea-pigs for animals, and transplanting human cancers, both of the epidermoid and glandular types, and transplanting tissue bits and emulsions both subcutaneously and peritoneally. There never resulted any tumor formation or growth of the transplanted cells. In some cases the nodule transplanted suppurated, in others it finally disappeared, leaving only scar tissue. Gaylord[2] inoculated guinea-pigs with peritoneal fluid from a case of human abdominal cancer, and found in the lung of one animal a small nodule which he considered to be adeno-carcinoma. In another similar case he inoculated a dog with peritoneal fluid and killed the animal after ninety days. He found numerous nodules in the liver which he considered new formations derived from the liver epithelium. There is no proof of the reproduction of the original type of cancer. Hansemann,[3] who had opportunity to study the tissue from both these cases, denied that the nodule in the guinea-pig was a true adeno-carcinoma, and stated that similar appearances could be seen in human lungs in chronic induration. He also declared that the nodules in the dog's liver were not primary nodules, but were nodules secondary to some overlooked primary focus.

Another fact which makes these reported successes theoretically improbable is the fact, as will be shown later, that in

[1] Dagonet. Transmissibilité du Cancer. C. R. Soc. Biol., iv, 1903.

[2] Gaylord.

[3] Hansemann. Verhandl. des Comités f. Krebsforschung, 1902–3. Heft, ii, p. 34.

some cases of cancer in rats where successful transplantation from one rat to another has been successfully and readily carried out, there has been absolute failure in all attempts to transfer the same tumor to animals of another species, *e.g.*, to guinea-pigs or rabbits.

On the whole, in regard to these experiments, it is fair to say that in the literature there is no proved or authentic case of the transfer of human cancer to another animal, and that the evidence in favor of the claim of such transference is in no case of such a character as to make the accuracy of the claim even probable.

7. *Inoculation of Cancer or Malignant Tumor into Another Animal of the Same Species.*

A considerable series of experiments showing the possibility of transferring cancer or other malignant tumors from one animal to another of the same species exists, and these results in several cases have been carefully verified by exact and painstaking work. Hanau[1] put tissue from an epidermoid cancer of a rat into the scrotum of another rat and got a similar epidermoid cancer at the point of inoculation, but had no metastases in the lymph nodes. Loeb[2] transplanted tissue from a cystic sarcoma of the thyroid of a rat to other rats, and produced at the point of inoculation a tumor of histological structure similar to that of the original tumor. These transplanted tumors infiltrated surrounding tissues, but did not form metastases. He could not inoculate the tumor into other species of animals. He showed that the new growth came from a proliferation of the transplanted cells and not from infection of the fixed cells of the inoculated animal, *i.e.*, that the new formed tumor was a true transplantation and not an infection. He also successfully transplanted into other rats tissue from a mixed tumor

[1] Hanau. Erfolgreiche experimentelle Übertragung von Carcinom. Fortschrit. des Med., 1889, ix, p. 321.

[2] Loeb. Transplantation of tumors. Jour. Med. Research, vi, p. 28. Also Uber Transplantation von Tumoren. Virch. Arch., clxxii, p. 172, 1903. Also Mixed tumors of thyroid gland. Am. Jour.Med. Sci., February, 1903, p. 243. Also Further investigations in transplantation of tumors. Jour. Med. Research, viii.

of the thyroid of a rat. Inoculation of rats with juice from the tumors after filtration to remove tumor cells gave no growth. In neither case could organisms of any sort be cultivated. Velich[1] transplanted tissue from a periosteal sarcoma of the thigh of a white rat to other rats, and at the point of inoculation got tumors whose structure constantly was like that of the original tumor. After some generations of success the power of being transplanted was lost. Inoculations with tumor juice from which cells had been removed failed. It is noteworthy that uninoculated animals kept in the same cage with inoculated animals developed similar tumors. In his experimental animals no metastases were formed. Morau[2] inoculated rats with an emulsion of tissue of a cylinder-celled adenoma of the axilla of a rat, and produced at the point of inoculation tumors with the same histological structure. The transplantations were carried through several generations, but the inoculalibility of the tumor finally diminished. No organisms could be cultivated. Borrel[3] inoculated rats through six generations with an epidermoid cancer of another rat. Transplantations succeeded in about ten per cent of the cases. In some of the inoculated animals metastases incurred with involvement of lymph nodes. The tumors appeared at the point of inoculation, and their histological structure was identical with that of the primary tumor. Rats kept with inoculated animals also developed cancers. Jensen,[4] in the most valuable contribution yet made to this subject, reports successful transplantation of skin cancer from a mouse to other mice. The inoculated animals developed tumors at the point of inoculation, the histological structure of which was identical with that of the original tumor. He carried successful implantations through nineteen generations, and succeeded in about one-third of the animals. He was unable

[1] Velich. Beitrag zur Frage nach der Übertragung des Sarkomes. Wien. Med. Wochenschr., 1898, p. 711.

[2] Morau. Récherches expér. sur la transmissibilité des certains néoplasmes. Arch. de Méd. expér. et d'Anat., September, 1894, p. 677.

[3] Borrel. Epithélioses infectieuses et epithéliomas. Ann. de l'Inst. Pasteur, February, 1903, p. 81.

[4] Jensen. Experimentelle Untersuchungen über Krebs bei Mansen. Centralbl. f. Bakt., xxiv, 1903, p. 28.

successfully to transplant to other species of animals. He saw no parasites in his experimental tissues, nor was he able to cultivate any organisms. He inoculated rabbits with tumor tissue from rats, and from the rabbits obtained a serum which in rats destroyed the tissue of experimental tumors. He inoculated rats with tissue from their own tumors, and was inclined to believe that he thus obtained immunity in the animals. He also believed that with serum obtained from animals inoculated with tumor tissue, and so made actively immune, he could protect rats from transplantation (passive immunity). Young tumors were less successfully transplanted, and Jensen believes that this is due to the fact that the rapidly mitosing cells of young animals cannot stand interference with their nutrition as successfully as the less rapidly growing cells of old tumors. None of Jensen's tumors formed metastases. Jensen showed that the tumors arose from proliferation of the transplanted cells and not from infection of the fixed cells of the inoculated animal, *i.e.*, that they were true transplantations. In a few animals endocellular inclusions were seen, but Jensen says that they were degenerations.

These experiments show the certain possibility of the transplantation from one animal to another of the same species of tumor tissue, both cancer and sarcoma, with formation of tumors at the point of inoculation of histological structure identical with that of the primary tumor. This transplantation can be carried through many generations, although not every individual experiment succeeds. In very few instances endocellular structures are seen, due to degeneration. In no case has any organism been cultivated from the experimental tumors. All observers agree that the tumors are true transplantations and that the tumors develop from proliferation of the transplanted cells and not from infection of the fixed cells of the inoculated animals. When tumor cells are excluded by filtering, in no case does the injected cancer juice cause tumors. In but few cases were metastases or lymph node infection produced.

8. *Parasitic Production of Epithelial Proliferation Analogous to the Epithelial Proliferation in Cancer.*

In support of the parasitic origin of cancer it has been claimed that various other diseases characterized by marked proliferation of epithelium are due to the action of a protozoon parasite. The diseases mentioned in this connection are coccidiosis, Darier's disease, molluscum contagiosum, and a class of diseases which Borrel includes under the term "epithélioses infectieuses," *i.e.*, clavelée or sheep pox, variola, varioloid, etc. Tyzzer[1] has shown that the primary effect of the coccidium oviforme is a destruction of epithelium with a secondary formation of connective tissue, which later becomes covered by epithelium, and that the real lesion is of the nature of a chronic inflammation. Darier's disease has never been proved to be of parasitic origin, and that opinion now is generally discredited. As regards molluscum contagiosum, White and Robey[2] have shown that no parasite ever has been demonstrated in the disease. As regards the "epithélioses infectieuses," although in these diseases there is a marked proliferation of epithelium, the proliferation is not analogous to the proliferation of epithelium in cancer. Borrel[3] has shown that the secondary nodules in the lungs in clavelée are derived from the fixed epithelial cells of the lung, while, as has already been shown, the secondary metastases in cancer are of an entirely different origin. Borrel says that the morphology of the cell inclusions in clavelée, etc., is not like that in cancer. Dr. Councilman assures me that the intracellular bodies in variola and varioloid are not analogous with those of cancer.

Summary of Conclusions from General Arguments for Parasitic Origin of Cancer.

From a consideration of the facts already presented it may be said that (1) the relative increase of cancer is disputed

[1] Tyzzer. Coccidium infection of the rabbit's liver. Second Ann. Rep. of the Cancer Committee, Harv. Med. School, Boston, February, 1902, p. 1; Journ. of Med. Research, vii.

[2] White and Robey. Molluscum Contagiosum, same reference, p. 23.

[3] Borrel. Loc. cit., supra.

and not proved; (2) local causes of the origin of cancer are improbable; (3) the metastases in cancer, although taking place by the same routes as the metastases in infectious diseases, are of an absolutely different origin, and constitute the strongest theoretical argument against an infectious or parasitic origin; (4) the cachexia in cancer probably is not entirely due to the character of the cancer itself, but is often dependent upon secondary bacterial infection; (5) there is no proof of the transfer of cancer from one human being to another by contact; (6) transplantation of cancer from one animal to another of the same species is well verified; (7) the production by any known parasite of epithelial proliferation analogous to cancer has not been demonstrated.

II. SPECIAL REASONS FOR BELIEF IN PARASITIC ORIGIN OF CANCER.

There occur in cancers, generally but not always within the cells, peculiar bodies, first described by Virchow, and considered by him to be evidence of endocellular cell division. These bodies are variously described, but the typical ones consist of a central dot surrounded by a relatively clear space, which in turn is surrounded by a membrane, often described as "double contoured." Of recent years these bodies have been closely studied, and, for various reasons, have been considered by some men to be parasites and the cause of cancer. These men believe that the bodies are parasites partly because of the supposed morphological resemblance to certain known parasites and partly because of what they believe to be an experimental production of cancers by inoculation of animals with the parasites. Some investigators believe that the bodies are blastomycetes or torulæ, others that they are protozoa.

1. *Blastomycetic Theory.*

The status of the theory that these bodies are blastomycetes and the cause of cancer can, in my opinion, be dismissed in a few words. The detailed reason for this

opinion can be found in the previous report by the Cancer Committee of the Harvard Medical School. In that paper I drew the following conclusions from an examination of the results of the experience of other men and of myself. Examination of the results of the experience of other men and of my own experiments lead me to the conclusion that:

1. Certain blastomycetes can live and multiply in human and animal tissues, produce local lesions and metastases in the internal organs, *i.e.*, they are pathogenic.

2. The lesions produced in animals by spontaneous infection with blastomycetes are acute inflammations, abscesses, or nodules of peculiar granulation tissue, and are not in the least analogous to cancers.

3. The lesions produced in human beings in cases of spontaneous infection with blastomycetes are acute inflammation (abscesses or ulcers), or proliferation of endothelium and connective tissue. At times a proliferation of the epidermis does occur, but is not due to the action of the blastomycetes, but is secondary to the chronic inflammation of the underlying corium. This proliferation of epidermis is not analogous to the proliferation of epithelium seen in cancers, since no epithelial metastases occur.

4. Blastomycosis in human tissues is very rare.

5. The lesions produced in animals by experimental inoculation with blastomycetes are, with the exception of Sanfelice's "successful" cases, inflammations or nodules of peculiar granulation tissue. Sanfelice's cases are not conclusive in themselves, are in direct opposition to the results obtained by all other observers, and, even if true, are logically explained as coincidences, and not as results.

6. Blastomycetes, as a rule, cause marked proliferation of tissue, and little infiltration with leucocytes, *i.e.*, their toxic powers are small.

7. Blastomycetes primarily extend along lymphatic clefts and vessels.

8. Rarely in human beings, more often in spontaneously infected animals, and often in experimental animals blastomycetes may be taken into the blood vessels, disseminated

throughout the body, and produce a general infection and metastases.

9. The secondary nodules have the same general character, *i.e.*, a formation of granulation tissue, as the original nodules.

10. The morphology of the so-called "cancer bodies" is not identical with that of the blastomycetes.

11. Blastomycetes are not constantly present in human malignant tumors and cancers.

12. Even if blastomycetes do occur in human cancers they are not present in such numbers and in such a relation to the anatomical lesion as to justify the belief that they are the cause of the disease.

All of these facts lead to the ultimate conclusion that there is no evidence that blastomycetes have anything to do with the production of human cancers.

The blastomycetic theory has rather lost its vogue of recent years, and in my opinion there has been no new evidence which would lead to any change of the above opinions regarding the relation of blastomycetes to cancer. On the other hand, since the appearance of my report, Foulerton and Price-Jones[1] have published a paper upon the bacterial examination of malignant tumors, which is in agreement with the results reported by Dr. Oscar Richardson in the second report of the Harvard Cancer Committee, while Young[2] has reported observations on the pathogenic action of blastomycetes which confirm the correctness of my own observations. Foulerton and Price-Jones examined fifty-six malignant tumors, and concluded that "none of the so-called 'cancer yeasts' stood in any casual relation to cancer." Young inoculated animals with the blastomycetes of Sanfelice, Plimmer, and others, and concluded that pathogenic blastomycetes produced in animals' nodules composed of granulation tissue and blastomycetes, and that, although occasionally blastomycetes could be seen within cells which resembled cancer

[1] Foulerton and Price-Jones. The bacterial exam. of cases of carcinoma and sarcoma. Rep. Cancer Research Lab., Middlesex Hospital, i, London, 1902.

[2] Young. The pathogenic action of blastomycetes. Rep. Cancer Research Lab., Middlesex Hospital, i, London, 1902.

bodies, they were not the same as cancer bodies either morphologically or in their staining reaction. Moreover, Malvez[1] has shown that the serum of animals inoculated with yeasts has the power of agglutinating those yeasts, but Brauha[2] showed that the serum of cancer patients did not agglutinate the cancer yeasts, as they should have done if blastomycetes were present in, and the cause of, cancer.

2. *Protozoon Theory of the Causation of Cancer.*

The evidence that protozoa are the causes of cancer can be divided into three heads. Some men believe that the cancer bodies are protozoa because of their alleged similarity to known protozoan parasites. Others have claimed to "cultivate" the alleged organisms, and to produce cancerous tumors by inoculating animals. Still others believe that a certain protozoon, the "plasmodiophora brassica," produces, in plants, cell changes analogous to the epithelial proliferation seen in cancers, and that therefore it is conceivable that the epithelial proliferation in cancer also may be due to a protozoon.

1. Morphological evidence of parasitic nature of cancer bodies.—Without going into a detailed review of the descriptions of the "parasite" by all of the different observers, a general statement of the conclusions drawn can be made. Thoma[3] described small unicellular organisms in the nucleus of epithelial cells of cancer, which from their size and shape and composition he believed undoubtedly were parasites, which might be the cause of cancer. Russell[4] described bodies, "fuchsin bodies," seen in the protoplasm of cancer cells, also in gummata, in fibro-adenomata, and in a "gelatinous sinus" of the knee. The bodies also were found in round cell infiltrations, in the connective tissue stroma, and in the

[1] Malvez. Centralbl. f. Bakt., xxix, 688, 1901.

[2] Brauha. Centralbl. f. Bakt., xxx, 945.

[3] Thoma. Über eigenartige parasitäre Organismen in den Epithelzellen der Carcinome. Fortschr. der Med., vii, 413, 1889.

[4] Russell. Brit. Med. Jour., 1890, ii, 1356.

lymphatics. Russell described the bodies as spheres having a refractile capsule and staining intensely with fuchsin. He said that the bodies reproduced by budding or by the formation of spores. Ruffer and Walker[1] described bodies which occurred in the protoplasm of cancer cells, spherical, having a central nucleus, surrounded by protoplasm and a refractile capsule, often double. Similar bodies were seen by Sjöbring,[2] who said that they formed spores and cysts. Ruffer and Plimmer[3] described similar bodies, but saw no cysts. Similar bodies were described by Sawtschenko, Podwyssozki, Steinhaus, Foa, Roncali, von Leyden, Bosc, Schueller, and many others. Some men, Roncali and others, claimed to find such bodies in sarcomata. A study of the descriptions by the various men shows that one factor is constant in all of the descriptions, *i.e.*, the presence of small spherical bodies composed of a center, a surrounding clear zone, and a refractile membrane, the latter often described as "double contoured." Beyond that point all agreement ceases. Some claimed that the bodies could be seen in the cell nucleus, but most agreed that the bodies came in the epithelial cell protoplasm. Some found them intercellular or in the connective tissue stroma or in lymph spaces. Some men believed that the bodies reproduced by budding, others that they formed spore cysts and spores. Some claimed to find "sickle bodies." Various life cycles of the so-called parasite were worked out, no two agreeing on the successive steps. Besides these typical bodies a vast variety of other inclusions have been described. In fact any bizarre figure seen in cancer cells apparently was assumed to be one of the protean shapes which the parasite could take.

While many observers thus agreed upon the general appearance of the cancer bodies, the interpretation of their nature and their position in the animal world was very divergent. Some observers believed them to be blastomycetes. The

[1] Ruffer and Walker. Parasitic protozoa in cancerous tumors. Jour. of Path. and Bact., i, 198.

[2] Sjöbring. Ein parasitärer protozoaartiger Organismus in Carcinomen. Fortschr. der Med., viii, 529, 1890.

[3] Ruffer and Plimmer. Jour. Path. and Bact., i, 395, ii, 3, 1892.

status of the blastomycetic theory has been stated. Others believed that they were animal parasites and that they belonged in some one of the various classes of the protozoa. This divergence of opinion shows conclusively that their morphology is not sufficiently constant to allow any agreement as to their exact nature.

Other men, after a careful study of these cancer bodies, came, on a morphological basis alone, to the conclusion that they were not parasites. Some men claimed that the bodies were a result of cell degeneration, and Pianese's valuable monograph shows clearly that many of the endocellular bodies can be explained in that way. Other forms have been ascribed to the inclusion of red blood globules in the cells, or of polynuclear leucocytes. Borrel[1] believed that the typical bodies represented centrosomes. Dean[2] believed that the inclusions were masses of hyaline degeneration. Völcker[3] demonstrated that Schueller's "parasites" were cork cells accidentally present. It is noteworthy that in Labbé's Tierreich[4] all of these alleged parasites of different observers are included under the term "pseudo-coccidia." Greenough[5] advanced a theory which seems to me to explain better than any other the peculiar form and character of the typical cancer bodies. Greenough observed that the bodies occurred only in cancers arising in glandular organs and believes they are due to secretory activity of the cancer cell. Tadaò Honda,[6] after a careful study of the cancer bodies, agreed that they came only in glandular cancer; that they were not parasites, and that they represented the secretion of a hyaline substance.

So, on the whole, it may be said that from a morphological study of the cancer bodies no agreement has been reached as to their nature or origin, nor on a morphological basis is

[1] Borrel. Ann. de l'Inst. Pasteur, xv, No. 2, 1900, 49.

[2] Dean. Dr. Russell's characteristic organism of cancer. Lancet, 1891, i, 768.

[3] Völcker. Dtsch. med. Wochenschr., July 25, 1901.

[4] Labbé. Tierreich. Sporozoa. Berlin, 1899.

[5] Greenough. Cell inclusions in cancer. Second Rep. Cancer Committee, H. M. S., p. 128., Jour. of Med. Research, vii.

[6] Tadao Honda. Zur parasitären Ätiologie des Karzinoms. Virch. Arch., clxxiv, 1903.

it likely that any proof of the parasitic nature of the bodies can ever be made.

2. Experimental and "cultural" evidence of parasitic nature of cancer bodies — Schueller[1] claimed that by taking bits of perfectly fresh cancer, in which he found so-called parasites, and placing them in sterile test-tubes which were kept at body temperature, an increase in the number of parasites could be detected. This cannot, even allowing that his observations of the bodies were correct, in any sense be considered evidence of "cultivation," and, as has already been mentioned, it has already been shown that some at least of his alleged parasites were cork cells. Gaylord[2] inoculated a guinea-pig with ascitic fluid from a case of abdominal cancer, and believed that he produced an adenoma of the lung; he also inoculated a dog with ascitic fluid from another case of abdominal cancer, and believed that he produced disseminated nodules of cancer in the animal's liver. Allowing that the results were such as Gaylord claimed, the experiments offer no evidence of inoculation with a "parasite," for the possibility that cancer cells were present is not eliminated. Moreover, as has already been shown, competent men who have had ample opportunity to study his sections deny the accuracy of his primary observations.

3. Evidence that known protozoa can produce in plants cell changes analogous to cancer in animals. — In cauliflowers and other members of the cabbage family occurs a disease called by the Germans "Kohlhernie," in English "club-root," which is due to an infection of the plant cells by a protozoon, the plasmodiophora brassica. The organism appears as spores in the cells of the affected plant, and it has been claimed that the lesions produced in plants by these spores are analogous to the epithelial cell proliferation in cancer, and hence by analogy that the cancer bodies are protozoa

[1] Schueller. Beitrag zur Ætiologie der Geschwulste. Centralbl. f. Bakt., xxvii, 14-15, p. 511, 1900.

[2] Gaylord. Loc. cit., supra.

and the cause of cancer. Animals have been inoculated with these spores to see if these known protozoa would produce epithelial proliferation. Podwyssotzki[1] inoculated rats and guinea-pigs, and produced "tumors" of mesodermal origin "similar to large-celled sarcomata or endotheliomata or the granulomata seen in leprosy," but did not produce epithelial proliferation. Some of the spores were seen in (phagocytic) cells. He thought that these endocellular spores looked like the inclusions seen in sarcomata (sic). Gaylord[2] inoculated animals with fresh club-root and produced nodules of granulation tissue, with but little infiltration with leucocytes after the twelfth day, containing many phagocytic cells in which were spores and spore cysts. No epithelial proliferation or cancer was produced. Gaylord[3] says that spores of the plasmodiophora brassica produce, in both cold and warm-blooded animals, appearances "indistinguishable from that of cancer inclusions." This must apply to the appearance of the spore itself, since the spores are seen only in phagocytic connective tissue or endothelial cells, and that is no evidence whatever of the parasitic nature of cancer bodies, for exactly the same statement as regards morphology can be made in regard to occasional faintly staining endocellular blastomycetes. Von Tubœf[4] has shown that the so-called "cancers" of plants may be caused by animal or vegetable parasites or by frost, and cannot be compared with human cancers. When due to a plant fungus the parasite is well known and of large size, and unlike the cancer bodies. He inoculated animals with plasmodiophora brassica and produced nothing analogous to cancer.

On the whole, as regards the protozoan theory, it may be said that a settlement of the question on a morphological basis alone is very unlikely, especially in dealing with a disease

[1] Podwyssotzki. La Presse Méd., 1900, 77.

[2] Gaylord. Plasmodiophora Brassica. 4th Annual Rep. Cancer Lab., N. Y. State Board of Health, 1902–3, 20.

[3] Gaylord. On the evidence of the nuclear division of certain cell inclusions. Loc. cit., 53.

[4] Von Tubœf. Ueber Krebs bei Pflanzen. Verhandl. des Komités für Krebsforschung. Heft, i, 74, Berlin, 1902.

the incubation of which is unknown, and the early stages of which so seldom can be studied. For, in dealing with microscopic spheres of not too constant form, it will always be possible to confuse them with, or see resemblances to, various simple lower forms of both plant and animal life which also have a spherical shape. Unless a definite and constant cycle of stages can be seen which closely resemble the life cycle of known vegetable or animal parasites, conclusions based upon morphology alone must always be doubtful, especially in a disease like cancer, the infectiousness of which is not proved. Lubarsch has said that for those who claim that the cancer bodies are parasites, it is necessary to prove not merely that the bodies resemble known parasites, but that they cannot be anything else. This statement is emphasized by the fact that certain observers have considered the cancer bodies at one time to be protozoa and at another blastomycetes, and vice versa. The constancy of form of the bodies is not such as to allow as yet any agreement upon their alleged life cycle.

Moreover, there is no proof that any known protozoon produces a proliferation of epithelial cells analogous to the epithelial proliferation in cancer. Nor has there been any verified, indisputable, or convincing proof of the production of cancer by any of the so-called protozoon cancer parasites. Nor has there been any successful isolation, cultivation, and inoculation of any cancer protozoon in accordance with Koch's law. As regards protozoon parasites it must be acknowledged that as yet it is practically impossible to present proof of their parasitic nature in the way that is demanded in the case of bacterial parasites, and certain diseases are accepted as due to a protozoon parasite without complete compliance with Koch's law, but in the case of the so-called cancer parasite the alleged cultural and inoculation experiments are so imperfect as to exclude even the probability of their being the cause of the lesions they are alleged to have produced. The same reasoning applies with redoubled force to the blastomycetic theory because it is a simple matter to cultivate blastomycetes outside of the body, and yet in no

case of alleged cancer production has Koch's law been complied with.

III. THEORIES AS TO THE BIOLOGICAL ORIGIN OF CANCER.

Opposed to those who believe that the epithelial proliferation in cancer is due to the action of a parasite are many men who believe that it is due to the development of inherent or acquired peculiarities of the cells themselves, and that the proliferation of the tumor cells can be explained on biological grounds. Various hypotheses have been advanced to explain the unlimited growth of the epithelium in cancer or the tissues of other malignant tumors, with the formation of metastases. The hypotheses which have received widest acceptance are those of Thiersch, of Cohnheim, and of Ribbert, to which may now be added the hypothesis supported by Bashford and Murray. Each of these hypotheses is supported by a greater or less number of clinical facts, and Cohnheim's and Ribbert's have been tested by a considerable amount of experimental work.

Thiersch[1] observed that some benign tumors, *e.g.*, warts, were composed of epithelial cells and a connective tissue stroma, and yet that the same elements entered into the structure of malignant cancers, in which infiltration of surrounding tissues by epithelium was the marked feature. He therefore decided that the mere presence of epithelial cells alone could not be accountable for the infiltrating peculiarity of cancers. He therefore assumed that a balance of the tissues in the body was maintained by a continual exercise of mutual pressure of one tissue upon another, and that the maintenance of this balance depended upon the perfection of the nutrition of each of the adjacent tissues. Hence if any given tissue had its nutrition interfered with it became unable to maintain its relative pressure, and so might be overwhelmed by adjacent tissues which retained their nutrition, *e.g.*, in old people for some inherent reason the connective tissue failed to maintain its nutrition, hence the resistance

[1] Thiersch. Der Epithelialkrebs. Leipzig, 1865.

of the tissue was lessened and an ingrowth of adjacent epithelial cells occurred. Hence the liability of old people to cancer. This hypothesis of Thiersch's seems to me hardly entitled to be designated as a "theory," but to be merely a restatement in general terms of a well-known clinical fact, *i.e.*, the infiltration of connective tissue by epithelial cells in cancer. Moreover, even if the hypothesis were a correct one, unless the real cause of the loss of nutrition could be determined, not the slightest light would be thrown on the cause of the disease. Moreover, beyond the general statement, the hypothesis fits but a small number of the known clinical facts.

Cohnheim's[1] hypothesis, however, has a much more impressive support by clinical and experimental facts. Up to Cohnheim's time great stress had been assigned to trauma as a cause of cancer. Cohnheim believed that no importance could be assigned to traumatism as a cause of the disease. It is perfectly well known by histological and experimental study what the reaction of tissue to traumatism is, *i.e.*, it leads to replacement of the injured tissue by granulation tissue, while the surface is covered by newly formed epithelium. Now to assume that trauma may cause cancer is to assume that the organism suddenly entirely changes the laws under which it ordinarily acts, and that, too, in an individual who previously has reacted to trauma in accordance with the regular laws many times. Moreover, many cancers develop in places in which no known trauma has occurred. A study of the cases of cancer in various clinics has shown that in a very considerable number of cancers no traumatism could be assigned. Although in some cases cancer is recognized soon after a traumatism, the relative frequency of such cases can be known only by knowing the entire number of traumatisms which occurred during the same period and the number of times that cancer appeared in that entire number. This number obviously would be exceedingly small. Hence the cases of cancer in connection with trauma either could perfectly well be explained as coincidences, or else some

[1] Cohnheim. Allgem. Pathologie. zweite anlage. Berlin, 1882, 732.

accessory cause other than trauma must be assumed to cause the sudden reversal of normal laws in reaction to injury.

Hence Cohnheim, declining to accept the theory of traumatic origin of cancer, assumed that in each of the primary embryonic layers more cells were produced than were necessary for the development of the adult tissues, and that some of these surplus embryonic cells did not develop into the normal tissues of the body, but persisted as "rests" which have the "potentiality of growth" characteristic of fetal cells, although they presented no gross or histological peculiarities which would permit of their recognition.

These supposititious "rests" once produced have an ultimate fate dependent upon various accessory conditions which may or may not act as a stimulus to their further development. It would be conceivable that an individual in whom these "rests" developed might grow to adult life and die without any accessory cause ever stimulating them to unlimited growth. Or the proliferation of these "rests" might begin at a very early stage of fetal life, and in this way the occurrence of congenital tumors could be explained. Or the accessory causes might become active during any period of life, early childhood, or young adult life, or the "rests" might remain quiescent during many years and be excited to growth only during later life, after, *e.g.*, according to Thiersch's idea, the normal resistance of some of the tissues had become lessened. Thus could be explained the occurrence of exostoses at the time of greatest bone activity or of ovarian cysts at puberty or of breast tumor during pregnancy or the occurrence of cancer in late adult life, all well-known clinical facts. The number of examples could be multiplied. Moreover, the constant occurrence of certain tumors in certain regions, *e.g.*, cancers of the lip or of the rectum, might be explained by such hypothetical "rests," as the production of such rests would be more likely to occur at such sites of active embryonic cell proliferation.

This "rest" theory also would explain the occurrence of heterologous tumors, *e.g.*, chondromata in bone, which differ widely from the normal tissue in which they occur. All of

the connective tissues are derived from the mesenchyma. Mesenchymal rests might be formed at any period of the development of these tissues. If they were stimulated to growth and continued to form cells of the period at which they were formed they might, *e.g.*, form tumors of cartilage in the midst of bone. In the same way certain sarcomata, *e.g.*, the myxosarcomata, resemble histologically certain stages in the development of normal connective tissue. By Cohnheim's hypothesis such tumors could arise from a proliferation of the fetal rests with retarded development.

The attractive part of Cohnheim's hypothesis is the fact that the same explanation serves to account for the occurrence of benign and malignant tumors. It also is in accord with a large number of known clinical facts. But although this hypothesis might be acceptable as explaining the origin of tumors, yet it in no way accounts for the cause of the sudden activity of these rests, which clinically may start up at any stage of intra or extra uterine life. To meet this objection Cohnheim suggested the possibility that an increase of the local blood supply, combined with a supposed diminished resistance of the adjacent normal tissues, due to a physiological limit of growth, might determine the sudden activity of these supposititious rests.

In support of Cohnheim's hypothesis is a certain amount of experimental evidence. Leopold[1] implanted both epithelial tissue and cartilage from fetal and adult animals. In many cases the implanted tissues disappeared or became encapsulated; in some cases they grew. Tissue from either young or adult extrauterine animals showed relatively little tendency to grow. Tissue from fetal animals showed much greater power of growth, and, roughly speaking, the younger the fetus the greater this tendency, and some fetal tissues by growth produced a nodule a thousand times greater than that of the original mass, and formed what Leopold considered a true "tumor." Fetal cartilage thus implanted showed great proliferation of cartilage cells, and also developed in some

[1] Leopold. Experimentelle Untersuchungen über die Atiologie der Geschwülste. Virch. Arch., lxxxv, 283, 1881.

cases into true bone. In no case did metastases appear, nor was there any evidence of infiltration of adjacent tissues. The transplanted tissues in some cases preserved the vitality and power of growth for very considerable periods. Zahn[1] implanted rib cartilage from adult animals, and found that the cartilage became encapsulated and did not grow. Implantations of fetal cartilage, however, showed marked growth of the cartilage cells. Cohnheim and Maas[2] put a piece of adult periosteum in the jugular vein of fowls, and found that these artificial emboli in the early stages showed active growth with formation of cartilage and bone, but after a few weeks all of the nodules were resolved and disappeared. Lack[3] opened the peritoneum of rabbits and scraped the ovaries, thus setting free ovarium epithelium. In one animal at the end of fourteen months there was found an undoubted columnar-celled infiltrating cancer of the uterus with a nodule infiltrating the diaphragm, and with metastases. On account of the great rarity of cancer in rabbits this case is particularly striking, but neither Lack nor others so far have been able to repeat the experiment successfully. Frænkel[4] implanted the placenta of rabbits into the same animal or into others, but got no results. He also implanted a rabbit with the sexual organs of her own fetus, and also repeated Lack's experiment, but in no case did any tumor result. These experiments show the possibility of transplanting certain fetal or adult tissues, entirely removed from their original environment, into other parts of the same animal or into other animals, and that in a certain proportion of the cases the transplanted tissue can maintain its integrity, grow, and reproduce the original type of tissue in some cases for an indefinite period. In but one case was any one of these experiments followed by a malignant infiltrating tumor with unlimited power of growth,

[1] Zahn. Sort des tissus implantés dans l'organisme. Congrès. period. internal de Genèvre, C. R., 1878, 658.

[2] Cohnheim and Maas. Zur Theorie der Geschwulstmetastasen. Virch., Arch., lxx.

[3] Lack. A preliminary note on the experimental production of cancer. Jour. Path. and Bact., vi, 154.

[4] Frænkel. Ueber Versuche, durch experimentelle verlagerung von Keim gewebe karzinomzu erzeugen. Centralbl. f. Allgem. Path., xiv, 664, 1903.

and this case, in consideration of the impossibility of repetition, must be considered as only a coincidence.

As further clinical evidence in favor of Cohnheim's hypothesis should be mentioned the occurrence of malignant tumors of the kidney composed of cells which reproduce in type the cells of the adrenal. The occurrence of such tumors suggests the probability that the tumors arise from an inclusion in the kidney of masses of adrenal cells during the embryonic period. Also the occurrence of syncitial tumors of the uterus, "deciduomata maligna," which appear in the uterus during or near pregnancy, are composed of cells which arise from the epithelial membranes of the ovum, which enter and infiltrate the uterus, remain for a time purely local, but ultimately become disseminated by the blood and form metastases in distant organs, is evidence in favor of the theory.

Ribbert's theory. — Ribbert,[1] from a histological study of very early cancers believes that the epithelial proliferation is due primarily to a separation of epithelial cells from their attachment to the normal epithelial layers by the action of the connective tissue. These freed epithelial cells, still retaining their power of growth, get into the clefts between the connective tissue cells, proliferate, and thus form islands of epithelial cells, which, freed from the normal restraining influence of other tissues, have an unlimited power of growth. There is some evidence, clinical and experimental, that normal epithelium, set free by accident or design from its original attachment, may retain its power of growth and produce nodules or cysts of epithelium, but there is no proof that true malignant tumors can be so produced, *e.g.*, repeatedly there has been reported the occurrence of cysts, "traumatic dermoid cysts," lined with epithelium, occurring in human beings, chiefly on the hands or face, subsequent to injury. It has been shown by histological study and by experiment that such cysts are due to accidental separation of surface epithelium, with implantation in the depths of

[1] Ribbert. Beiträg zur Histogenese des Carcinoms. Arch. f. Anat. u. Physiol., cxxxv, p. 433, 1894. Also, Zur Entstehung der Geschwülste. Dtsch. Med. Vochenschr., July 23, 1896.

a wound. Such cases have been reported by Wegner,[1] Bohm,[2] Le Fort,[3] Garré,[4] Blumberg,[5] Paulet,[6] Reverdin,[7] and Gironde.[8] Similar lesions have been seen in the iris and vitreous of the eye after injuries to the cornea. On the hands or face the nodules appear either as masses of pearly epithelium or as cysts lined with epidermis containing sebaceous material. The walls consist only of epidermis as a rule, and do not contain hair follicles or glands. Schweininger[9] and Kauffman[10] produced such cysts by implanting small bits of epidermis in the tissues of animals, but found that while at first the rate of growth was very rapid, after a time the rate diminished and the increase in size was very slow, *i.e.*, they produced epidermoid cysts which did not have the essential character of malignant tumors.

On the whole, in regard to the biological theories of the origin of malignant and benign tumors as enunciated by Thiersch, Cohnheim, and Ribbert, it may be said that such hypotheses, or a combination of them, is at present supported by a considerable number of clinical and experimental facts; that certain tumors, dermoid cysts, can be produced experimentally under the conditions of the hypotheses, and that in one doubtful case a truly malignant tumor appeared, and finally that fetal tissues undoubtedly have a much greater inherent power of growth than do adult tissues.

Too lately to allow of verification by other men, certain observations have been made which give support to the

[1] Wegner. Beiträg zur Lehre von den traumatischen Epithelcysten. Ref. Centralbl. f. Allgem. Path., x, 581, 1899.

[2] Bohm. Traumatische Epithelcyste und Fremd-körper Riesenzellen in der Haut. Virch. Arch., cxliv, 276.

[3] Le Fort. Des Kystes Dermoïdes Traumatiques. Révue de la Chir., 1894, 1013.

[4] Garré. Ueber traumatische Epithelcysten der Finger. Beiträge zur klin. Chir., 1894, xi.

[5] Blumberg. Ueber sogennante "traumatische Epithelcysten." Dtsch. Zeitschr. f. Chir., xxxviii, 605.

[6] Paulet. Des kystes dermoïdes acquis. Bull. et Mém. de la Soc. de Chir., Paris, xii, 460, 1886.

[7] Reverdin Des Kystes Épidermiques des doigts. Rev. Méd. de la Suisse romande, 1887, 121.

[8] Gironde. Contributions à l'étude des kystes dermoïdes. Lyon Medical, 1879.

[9] Schweininger. Beitrag zur Erzeugung Hautgeschwültzen. Charité Annalen, xi, 642.

[10] Kauffman. Ueber Enkatarraphie von Epithel. Virch. Arch., xcvii, 236.

theory that cancers may be considered to be a parasitic individual engrafted on a normal individual, and that they are produced by the conjugation of cells in a way analogous to conjugation of sexual cells which produce a normal individual. Farmer, Moore, and Walker[1] investigated cancers, and concluded that the reduction in the number of chromosomes in the mitosing cells took place in the same way as in the ripening of the sexual cells of animals and plants, and that the number of these chromosomes were half as numerous as in the somatic cells. They studied not only human cancers, but also cancers in various lower animals. Bashford and Murray[2] confirmed these observations. These observations show a series of changes in the nuclei of malignant tumors throughout the whole extent of their known zoological distribution parallel to those characteristics of the maturation of the sexual elements of the metazoa. If these observations are confirmed, it will be a most important step in elucidating the origin and development of tumors.

My own implantation experiments were undertaken bearing in mind the above presented evidence, except that since published by Bashford and Murray, with the belief that evidence of the biological origin of tumors far outweighed that for a parasitic origin. The experiments were undertaken to see if by any means normal epithelium or other tissue, either fetal or adult, freed from its original environment, could maintain its power of growth, and lead to the formation of anything which could be considered analogous to a tumor, *i.e.*, tissue with unlimited power of growth. About ninety animals in all were used, but only those animals in which no suppuration occurred are reported.

The transplantations were all performed under strict surgical asepsis, the hair being previously removed by the application of "Sulphur Starch" as a depilatory. The animals, except a few which died of various infections, were killed at

[1] Farmer, Moore, and Walker. Resemblances exhibited between the cells of malignant growths in man and those of normal reproductive tissue. Proc. Roy. Soc., Vol. lxxii.

[2] Bashford and Murray. Loc. cit., supra.

various times, usually some weeks or months after the operation, with the idea of giving the transplanted cells as long a time as possible for growth. Only rabbits and guinea-pigs were used. In some groups of experiments absolutely negative results were obtained. In others, chiefly with epithelium of the skin, nodules many times the size of the original implantations were obtained, analogous to dermoid cysts, but in no case was a true tumor or metastasis produced.

The experiments fall into groups in accordance with the tissue used.

1. *Implantation of Testicular Epithelium.*

Five animals. In each case the peritoneal cavity was opened, the testis was drawn into the abdominal cavity, the tunica was split, and the testicular substance was curetted and distributed in the peritoneal cavity. The peritoneum was then closed. The animals were killed at periods varying from sixty to one hundred and sixty-one days. In no case was there any evidence of growth of testicular epithelium. The peritoneum in each case was normal except for slight adhesions.

2. *Implantation of Ovarian Epithelium.*

Seven animals operated upon. In three cases the peritoneum was opened, the entire ovary, or a half of it, was removed and immediately imbedded in the subcutaneous tissue of the abdomen of the same animal. In each case, for about two weeks, a nodule, larger than the implanted ovary, probably from exudation, was seen in the abdominal wall. The nodule gradually diminished in size and finally disappeared. One animal was killed at the end of sixty-three days, one after seventy-one days, and the other after one hundred and one days. In all cases the only evidence of the implantation was a small spherical mass of dense fibrous tissue. On histological examination no remnant of ovary was to be found, the mass consisting of dense fibrous tissue. In four cases Lack's experiment was repeated, *i.e.*, the abdomen was opened and the ovary crushed or curetted, and the tissue disseminated

as widely as possible in the peritoneal cavity, and the peritoneal cavity was closed. The animals were allowed to live sixty-one, sixty-seven, ninety-three, and two hundred and ten days respectively. At autopsy no growth or other change in the peritoneal cavity was found beyond slight fibrous adhesion.

3. *Implantation of Renal Epithelium in the Same Animal.*

Four animals were operated upon. In each case a lumbar incision was made, the kidney was drawn out of the wound, and a small bit of the kidney cortex was excised without opening the pelvis of the kidney, and the tissue was immediately buried in the subcutaneous tissue of the same animal. The animals were killed at the end of three, sixty-seven, sixty-eight, and seventy days respectively. In the animal killed at the end of three days the kidney tissue maintained its contour, but showed marked surrounding infiltration with polynuclear leucocytes. The cells of the renal tissue were largely necrotic. In the other three cases the nodules at first rapidly increased in size, probably from the presence of exudation, and then gradually diminished and showed finally only a small spherical mass of dense fibrous tissue, in which, on histological examination, no renal tissue was to be found.

4. *Implantation of Liver Epithelium in the Same Animal.*

Ten animals were operated upon. In two cases a small piece of liver was removed, the liver wound sutured, and the removed liver crushed in a mortar in sterile salt solution at body temperature, and immediately injected into the subcutaneous tissue of the same animal. The animals were killed at the end of one hundred and twenty and one hundred and sixty-three days, and showed nothing. In two cases a similar emulsion was injected into the mesenteric vein of the same animal. The animals were killed at the end of one hundred and twenty-four and one hundred and sixty-three days, and showed nothing. In two cases in pregnant animals, at about half term, the uterus was opened, the fetuses removed, and

from one, while still attached to the placenta, a small portion of the fetal liver was removed and buried in the subcutaneous tissue of the mother. The uterus then was closed. The implanted nodules, after the disappearance of the exudate, slowly diminished in size, and finally disappeared, showing no hepatic tissue at autopsy. In four cases a small bit of adult liver was implanted in the subcutaneous tissue of the same animal with no result.

5. *Implantation of Adrenal Tissue into Kidney of Same Animal.*

Six animals were operated upon. The abdomen was opened, the adrenal was removed and implanted either in part or entire into the cortex of the kidney of the same size, and the kidney capsule sewed over it. The animals lived from four to eleven days. In each case the adrenal tissue was necrotic, showed some infiltration with leucocytes, as did adjacent renal tissue, and in the cases which lived longest the beginning of connective tissue encapsulation was seen, but in no case was there any evidence of adrenal proliferation.

6. *Implantation of Adult or Fetal Epidermis into the Same Animal.*

Eleven animals were operated upon, ten with adult, one with fetal epidermis. Rabbits were used entirely. A very small bit of epidermis from the ear, about as large as the head of a pin, was removed with fine scissors, and care was taken not to get corium. In most cases no hemorrhage occurred. This epidermis was immediately pushed into the subcutaneous tissue of the ear on the outer side of the cartilage. In this way the gross changes could be fairly closely observed on account of the transparency of the ear. In the course of a few hours the nodule assumed a more or less spherical form from exudation of hemorrhage, and increased in size. In the course of ten to fourteen days the nodule became smaller and white. Then came a fairly rapid increase in size, the spherical shape being retained, and then, in two

or three weeks, the nodules either increased in size slowly or remained of the same size. The animals were killed in periods varying from seven to two hundred and seventy days. On section the nodules showed either a firm consistency, or appeared as small cysts containing a whitish or yellowish grumous material. The larger nodules were perhaps twenty times as large as the piece originally introduced, but in all cases there was a marked increase. On microscopic examination it was seen that in every case the epithelium had proliferated and produced a spherical nodule of epithelium or a spherical cyst lined with epithelium. When the nodule was solid the entire mass was composed of epithelium, the inner layers being composed of laminæ of cornified epithelium, while the outer layer of cells was the same as the deeper layer of cells in normal epidermis. Between the inner and outer layers the layers of normal epidermis were reproduced. In the larger nodules, the true cysts, the center was a more or less spherical cavity lined with epidermis, the outer layer of which was like the deepest layer of the normal skin, while the innermost layer was composed of stratified cornified epithelium. The central cavity was filled with material like that seen in a wen, *i.e.*, granular material and broken down cornified epithelium, with, in some cases, hairs. In many of the cysts hair follicles and sebaceous glands can be seen in the wall of the cyst, but in no case are such follicles or cysts seen about the entire circumference, showing that epidermis had grown across the surface of the small wound of implantation, thus producing the spherical form, but that the new epidermis did not specialize to form hair follicles or glands, *i.e.*, the epidermis acted like the epidermis of a superficial wound. In all cases the nodule or cyst was surrounded by a fairly distinct layer of dense fibrous tissue. In no case was there produced any isolated nodule of epidermis, nor was there any evidence of infiltration of the surrounding connective tissue, nor was there any extension of epidermis along lymph sinuses to adjacent lymph nodes. In one case epidermis from a fetus at about half term was used, and immediately implanted into the subcutaneous tissue of the ear of

the mother. In this case the resulting nodule was relatively larger than in those cases where adult epithelium was used, but the histological picture was identical with that produced by the implant of adult epithelium. In many cases proliferation of the epidermis was rapid, as was shown by frequent mitotic figures.

So it will be seen that by these experiments nodules or cysts were reproduced, analogous to the "traumatic epithelial cysts" already mentioned, and also analogous to dermoid cysts, but in no case was a true infiltrating tumor produced. In many cases the "potentiality of growth" was retained by the epithelium to a marked degree, especially so when fetal epidermis was used.

7. *Implantation of Adult Uterine Epithelium, Pregnant and Non-pregnant, into the Same Animal.*

Ten animals were operated upon. In three cases the animal was pregnant when the transplantation was done. In seven animals the uterus was in normal condition. The abdomen was opened. If the animal was pregnant the uterus was opened and the fetuses were emptied out, together with the placental attachments. A cross section of the uterus, one-half centimeter long was then removed, and immediately implanted into the abdominal subcutaneous tissue of the same animal. The piece removed was in no case through the placental attachment. The uterus was then closed by suture, as was the abdominal wall and the wound of implantation. The nodule was of considerable size, and its gross changes could be followed. There came in each instance a marked increase in size from exudation. This first increase in size then gradually diminished, and the nodule either finally disappeared or, after two or three weeks, gradually increased again, until in one instance it attained a size ten times as large as that of the original nodule. On gross examination the nodules were generally irregular and rather elastic. On section the nodule was invariably cystic, either showing one cyst cavity, filled with a cloudy yellow fluid, or in some cases showing multiple cysts. On histological examination the

appearances were fairly constant within certain limits of variation dependent upon the rate of growth of the transplanted tissue. The cyst cavity in every case was lined with epithelium more or less closely approximating that of the normal uterine mucous membrane. In all cases the number of mitoses was small. In the smaller tumors the epithelium was almost columnar, and cilia were distinct. In the larger cysts the epithelium was more or less flattened, and cilia were indistinct. The walls of the cyst were in all cases irregular in outline, varying from coarse polypoid projections to complicated projections, giving a picture like that of a multilocular cyst. In some cases but one cyst cavity was seen, corresponding to the lumen of the transplanted horn of the uterus. In other cases several cavities of large size were seen, each as large as the cavity of the transplanted uterus. In one case where the transplanted tissue was taken from a pregnant uterus there appeared on one side of the resulting cyst very marked hyperplastic endometritis of the mucus forming a hemispherical nodule on one side of the cyst, the rest of the cyst being lined with a single layer of epithelium.

Directly outside the mucous membrane in most cases was a layer of loose connective tissue, with in most cases considerable infiltration with lymphoid cells (submucous connective tissue). Outside of this layer in many cases was a layer of smooth muscle fiber, corresponding to the normal smooth muscle of the uterus. In some cases this muscle was almost perfectly preserved, and showed practically no formation of scar tissue. In other cases this smooth muscle layer was less than normal, and in it there were extensive areas of dense connective or scar tissue. In other cases this muscle layer was extremely thin, due in many cases apparently to the great dilatation of the cavity of the transplanted uterus. About some of the larger cysts in some of the nodules no smooth muscle at all was to be seen. This probably is due to the fact that the epithelium of the mucous membrane acted as does surface epithelium when transplanted, *i.e.*, it grows over the surface of the wound of implantation, and in that way forms a cavity lined with epithelium. Since the growth of

epithelium is irregular, the nodule on section shows adjacent cavities which at first give the appearance of a multilocular cyst.

Outside of the muscular layer in all cases comes a layer of fibrous tissue, in some cases wide and clearly marked and composed of dense scar tissue and forming practically a capsule; in other cases, indistinct, and distinguished with difficulty, if at all, from the normal loose connective tissue, and forming no encapsulation at all. In no case was there any evidence of infiltration of adjacent tissues by the uterine epithelium, nor any evidence of metastases by extention along lymphatics to adjacent lymph nodes. The transplanted nodules in all cases maintained their " potentiality of growth," as shown by occasional mitoses and gradual increase in size for considerable periods of time, but nothing comparable to a malignant tumor was produced.

8. *Implantation of Placental Tissue into the Same (Maternal) Animal.*

This series of experiments was undertaken to see if anything analogous to the deciduoma malignum could be produced. Besides the attempts to transplant placental tissues direct, in all of the preceding class of uterine implantations, whenever the animal was pregnant the uterus was manipulated and massaged to see if it were possible to produce any absorption of placental tissue by the uterine blood vessels which would lead to placental metastasis. In no case did any such metastasis occur. In six animals direct implantations of placental tissue into the subcutaneous tissue of the same animal were undertaken. In three of these cases the uterus was opened late during the animals' pregnancy, when the fetuses were almost at term, and small bits of placenta were removed and immediately implanted into the mothers' subcutaneous tissue. In all three cases the result was the same. The nodule persisted for a time, and showed the usual early increase in size from exudation, with a later gradual diminution and final disappearance of the nodule. In three cases the uterus was opened very early in the pregnancy,

before distinct development of fetal outline, and the entire placenta was implanted in the mother's subcutaneous tissue. In each case the nodule ultimately disappeared.

9. *Implantation of an Entire Fetus into the Same (Maternal) Animal.*

Two animals were operated upon. In both cases the uterus was opened early in the pregnancy, and the fetus, about one-half centimeter long, was removed from its placental attachment, and with the yolk sac adherent immediately implanted in the mother's subcutaneous tissue. In one case the nodule ultimately disappeared. In the other case the fetal tissues retained their power of growth and the nodule gradually increased in size and, at the end of ninety days, produced a nodular mass at least ten times the bulk of the original fetus. On section this nodule showed several small irregular cysts containing clear glairy fluid, and the cysts appeared not to connect with each other. On histological examination a very complicated picture was found, analogous to that of a complicated teratoma. No definite fetus could be distinguished, but there was a remarkable mixture of cysts lined with epithelium, cysts due to necrosis, and areas of bone and cartilage. The entire mass of transplanted and growing tissue was surrounded by a layer of dense fibrous tissue. In places this fibrous tissue layer was thin and hardly to be distinguished from the normal subcutaneous connective tissue. In other places it was thick and dense. One of the larger cavities of the mass was due to necrosis of a considerable portion of the nodule. The walls of this cavity were composed of granulation tissue. The contents was necrotic tissue, the character of which could not be determined, with serum and many polynuclear leucocytes. Other cysts were lined with epithelium and glands like the lining of the intestinal tract, outside of which was a layer of lymphoid tissue, outside of which again was a layer of smooth muscle fiber. Of these fibers only one layer could be distinguished. Many of the epithelial cells lining the cysts were beaker cells, and in all cases the glands of the inner

walls of the cyst were like the glands of the normal intestine. In places were also large islands of hyaline cartilage, scattered irregularly about, and in other places were small areas of bone with trabeculæ and marrow spaces filled with marrow. The remarkable feature about this nodule is the fact that in a normal fetus the size of this implanted one the intestinal tract is represented by a tube lined with a single layer of columnar epithelium with no differentiation of glands or muscle, and, moreover, no differentiation of the mesenchymal tissues into bone has taken place, while in the nodule, produced by implantation, tissues closely resembling adult intestine and adult bone and cartilage have been produced. The nodule was slowly increasing in size at the time the animal was killed in order to complete this report.

10. *Implantation of Fetal Cartilage into the Same (Maternal) Animal.*

But one animal was operated upon. A pregnant rabbit, with fetus at about half term, was operated upon by opening the uterus, removing a fetus, and immediately transplanting a small bit of the cartilage of the femur, in which no ossification had taken place, into the subcutaneous tissue of the mother. As usual the nodule increased, grew smaller, and then gradually increased in size until a nodule more than ten times the size of the original nodule was produced. Histological examination showed an oval nodule, surrounded by a distinct capsule of dense fibrous tissue. Inside this capsule was a nodule partly bony, partly cartilaginous. The periphery was composed of a layer of fibro-cartilage. Inside of this layer was a layer of hyaline cartilage with very large cartilage cells, occurring singly and not in large groups. Inside of this hyaline cartilage, and occupying about one-third of the entire nodule, was an area of true bone, with trabeculæ in which were cells in transition stage between cartilage cells and bone corpuscles. The marrow was very vascular. The transition from bone to cartilage was much like the series of changes which take place at the epiphyseal line in the ossification of the long bones.

CONCLUSIONS.

1. Certain types of epithelium (epidermis), both adult and fetal, can be experimentally removed from their normal position and implanted into another part of the same animal, and under those circumstances can maintain their "potentiality of growth," retain their own peculiar character, and produce nodules analogous to dermoid cysts or more complicated teratomata.

2. In no case has any epithelium of a highly differentiated function been seen to maintain its power of growth or to proliferate.

3. The "potentiality of growth" is greater in the case of fetal than it is in that of adult tissues.

4. In no case has any infiltration of surrounding tissue by the transplanted epithelium been seen, nor any tendency to epithelial metastases.

5. Certain fetal connective tissues (cartilage) can be transplanted in the same way as epithelial tissues, and retain their "potentiality of growth."

6. Transplanted fetal tissues do not reproduce the stage of development at which they are transplanted, but tend to reproduce the ultimate stage of their normal development.

DESCRIPTION OF PLATES.

PLATE XII.

FIG. 1. Transplantation of adult epidermis into subcutaneous tissue of outer surface of ear of rabbit. Vertical section through rabbit's ear. Shows surface epidermis and hair follicles, thickened subcutaneous tissue in which is epithelial mass, A. Center of this circular mass consists of cornified epidermal cells, surrounded by a layer of epithelial cells like those of normal epidermis.

FIG. 2. Transplantation of adult epidermis. Vertical section through rabbit's ear. Shows early stage in cyst formation. Transplanted skin in crescentic fold, the inner side of crescent corresponds to the lower surface of the transplanted piece, and shows that some hair follicles were transplanted. The external surface of the crescent covered with smooth epidermis, which grew from points A, A, on the surface of the wound of

transplantation, thus bringing cornified epithelium against cornified epithelium. At B B some of this cornified epithelium has softened and dropped out in cutting, showing beginning of cyst cavity.

PLATE XIII.

FIG. 3. Transplantation of adult epidermis. Vertical section through rabbit's ear. Irregular cyst cavity surrounded by well-defined zone of dense scar tissue. One side of cyst shows evidence of hair follicles; upper surface shows no such structures. Wall of cyst shows thick epidermal layer like that of normal skin. Center of cyst contains desquamated cornified epithelial cells and hairs.

FIG. 4. Transplantation of adult epidermis. Vertical section through edge of cyst shown in Fig. 2. Drawn with high power. Shows character of cells composing wall of cyst. Mitotic figures present.

PLATE XIV.

FIG. 5. Transplantation of adult uterus into breast tissue. Vertical section through abdominal wall of guinea-pig. Three cyst-like cavities lined with epithelium represent the lumen of the uterus. Surrounding these cysts is a distinct layer of smooth muscle fibers. The large cavity below the uterine tissue is a breast cyst.

FIG. 6. Transplantation of adult pregnant breast into subcutaneous tissue of abdomen of guinea-pig. Vertical section through abdominal wall of guinea-pig, epidermis and corium removed, abdominal muscles present. Lumen of uterus surrounded by thin lay of scar tissue. Smooth muscle of uterine wall preserved, barbed hyperplasia of uterine mucosa on one side of lumen of uterus producing a polypoid projection into lumen.

PLATE XV.

FIG. 7. Transplantation of adult pregnant uterus. Vertical section through abdominal wall of guinea-pig. Epidermis and corium present, abdominal muscles removed. Multilocular cyst, representing lumen of transplanted uterus. Moderate layer of scar tissue surrounding cysts. Cyst A shows that smooth muscle fibers are retained. About the other cysts muscle layer is very thin or absent.

FIG. 8. Transplantation of adult uterus, non-pregnant, into abdominal wall of guinea-pig. Cyst cavity surrounded by thin capsule of dense scar tissue, surrounded by fat tissue. No remnant of smooth muscle fibers. Probably means that uterine mucosa covered in the wound of transplantation just as epidermis of superficial wound or as transplanted epidermis does.

PLATE XVI.

FIG. 9. Transplantation of fetal cartilage into abdominal wall of guinea-pig. Vertical section through abdominal wall. Transplanted cartilage

surrounded by capsule of dense scar tissue. Center of nodule shows ossification like that seen in normal epiplupeal line. Outer layers are composed of cartilage or fibro-cartilage.

FIG. 10. Transplantation of entire fetus into abdominal wall of mother guinea-pig. Vertical section through abdominal wall; A, A, cyst cavities lined with intestinal glands; B, island of cartilage.

FIG. 11. Transplantation of entire fetus into abdominal wall of mother; A, A, A, cyst cavities lined with intestinal glands of adult type; B, island of cartilage.

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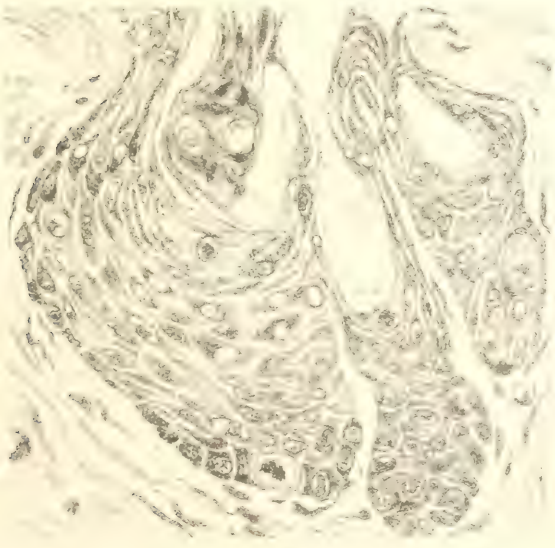

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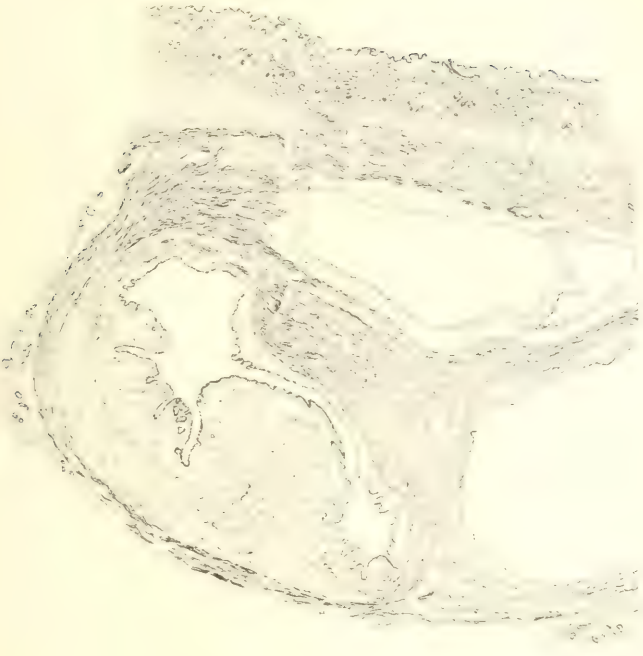

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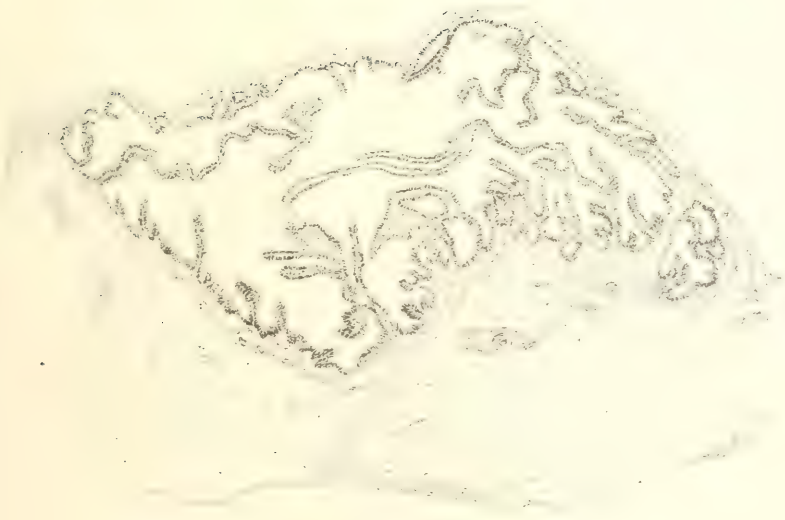

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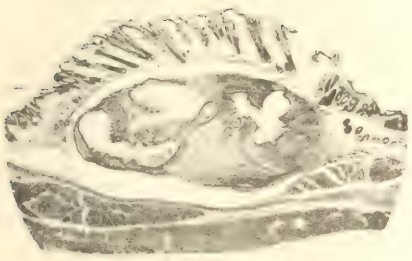

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